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Factors Affecting Odor in Beet Sugar

E. H. HUNGERFORD

The quality of commercial beet sugar generally has improved during the past 25 years. The improvement is most marked in respect to color, solution color, and ash content. Many factors, no doubt, have contributed to this improvement but the most important one is, probably, the practice of boiling higher purity pans.

Undoubtedly the odor of beet sugar has also been reduced during the past 25 years but since we have no absolute measure of odor the degree of improvement can only be conjectured. One's memory of an odor is very unreliable.

During the 1954 campaign, a preliminary survey was made to get some "leads" on factors affecting odor in white sugar. The problem was found to be illusive. In some instances, treatment of centrifugal wash water appeared to cause improvement. In other cases water treatment was without effect. Similarly, such diverse factors as weed roots, excessive amount of beet tops, badly deteriorated beets the venting of evaporators, and the washing of high raw sugar were investigated. Conflicting evidence was obtained.

Perhaps the most definite information obtained in a 1954 survey was on the subject of lime addition. In most, but not all cases studied, the odor of sugar from Steffen factories was found to be lower than that of sugar from the non-Steffen factories. Inasmuch as the lime addition at the Steffen factories is nearly double that at the regular beet houses, this would appear to be evidence that higher lime addition is effective in reducing sugar odor.

During the 1955 campaign, a planned study of the more promising leads uncovered in the 1954 survey was made. In this study the following factors were studied:

1. Centrifugal wash water treatment.
   (a) Untreated, (b) treated with ion exchange resin,
   (c) treated with carbon.
2. Washing high raw sugar.
3. Washing low raw sugar.
   (a) Return of low raw sugar to first carbonation.
   (b) Return of low raw sugar to high raw pans.
   (c) Return of low raw sugar to white pans.
4. High lime additions (at two Non-Steffen factories).
5. Higher purity pans.
6. Primary sources of factory water.
7. Miscellaneous sources of odors—dust boxes, sumps, etc.

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In order to compare sugar samples for odor it was necessary to set up standards. These were arbitrarily selected sugars believed to cover the range of odors which might be expected. The number of standards was subsequently reduced to two. A panel of testers from the research laboratory was able to differentiate between these standards. It was also able to score a large number of selected samples with reasonable agreement. Later all factory chief chemists were given an opportunity to test the procedure and to rate the same samples. Again, agreement was good. The procedure was simple. The samples were placed in clean, one-quart jars. Odor comparisons were made at about 30° C.

Treatment of centrifugal wash water with a cation exchanger resin or a mixed bed exchanger, followed by treatment with a granular carbon, usually caused a reduction in the odor of the water. This was particularly noticeable in the case of third and fourth evaporator body drips when the odor of the untreated water was distinct. In other cases the effect of treatment was less prounounced. Treatment of city water, which is commonly used for centrifugal wash, with mixed bed ion exchanger and granular carbon also reduced the odor but to a lesser degree.

In laboratory tests, factory massecuites were centrifuged and washed with treated and untreated water. In one series of tests the odor of the sugar was reduced by washing with treated water. In all other tests, the effects were too small to be measured with certainty.

Facilities for treating centrifugal wash water with cation exchange resin are available at three factories. At these factories the sources of the water are the third and fourth evaporator drips. Tests were made in which all the sugar produced during two consecutive days was washed with untreated water. Samples of sugar were taken during the second day of the test for comparison with sugars washed with treated water. Differences in odor of sugars washed with treated water and those washed with untreated water were too slight to measure.

In the preliminary survey of 1954, it was noted that high raw sugar carried a heavy odor and that this odor could be markedly reduced by adequate washing. Some work was done at the commencement of campaign in 1955 to determine the proper amount of wash water to use to reduce the odor to tolerable levels. It was found that, in general, about eight quarts of water are adequate. Actually the odor level of the high raw with this amount of wash water was only slightly higher than that of white sugar.

Similarly, the effect of washing low raw sugar on odor was determined. No attempt was made to reduce the odor of the low raw sugar to that of the washed high raw. About two quarts per machine were found sufficient to remove most of the molasses odor.

All of the washed high raw sugar was returned to standard liquor, that is to thick juice and melted sugar. Because of sulfate problems, it was found inexpedient to return all of the washed low raw sugar to standard liquor. During the two-week test period only about 35 percent was added to standard liquor, the remainder returning to first carbonation. Sugar produced during these test periods was compared with sugar produced

during periods in which the high and low raw sugars were not washed with optimal amounts of water. No measurable improvement in the odor of the sugar was obtained.

At two non-Steffen factories chosen because they represented two extremes of beet purities, the lime addition was varied over comparatively wide limits. At factory E which normally uses 1.5 to 1.7 percent CaO on beets, the lime addition was raised during two two-week periods to 2.17. At factory A which normally uses 1.35 to 1.4 percent lime on beets, the lime addition was increased to 1.80 percent. At neither factory was the odor of white sugar significantly improved by the larger lime addition.

An attempt was made at one factory to determine the effect of boiling higher purity white pans on the odor of the sugars. Because of the abnormally low purity beets encountered during this campaign, it was found impossible to maintain slicing capacity and hold white pans as high as was desired. However, during a brief period when the purities were held somewhat higher than normal, the sugar produced showed only trivial improvement.

Investigation of factory water supplies with respect to odor disclosed that at two factories the factory water supplies did, indeed, carry considerable odor. Odor of sugar produced at these factories was not higher than that of other comparable factories.

Other miscellaneous possible sources of odor were investigated. Sumps occasionally showed some odor. Dust boxes and melters were surprisingly free of odor this year.

The results of the special study appear to be essentially negative. A reason for this lies in the fact that the entire level of odor is lower in 1955 than it was in 1954 and probably lower than in several years. Table 1 shows a comparison between the odor in white sugar produced in 1954 and in those produced in 1955. The numerical values are arbitrary but should indicate factually the true difference.

Table 1.—Comparison of Odor in White Sugar Produced in 1954 and 1955.

Factory (non-Steffen)	Odor		Factory (Steffen)	Odor	
	1955	1954		1955	1954
A	0.7	3.0	J	0.6	2.0
B	0.7	4.0	K	0.9	1.3
C	0.5	1.0	L	1.4	5.0
D	0.6	2.3	M	1.5	2.0
E	0.3	2.0	N	1.0	1.3
F	0.6	2.0	Q	1.1	2.0
G	0.8	2.0			
H	0.7	3.3			
I	0.8	2.0			

It is difficult to account for the great differences in the odor level in the two years. Beet purities were generally lower in 1955 than in 1954. Sugar content was also lower in 1955. Topping of beets was probably neither better nor worse in 1955 than in 1954. There was little evidence of serious deterioration of beets in piles in either year. Because of the low purity of beets, more high purity sugar had to be introduced in white pans to maintain normal white pan purities. This may have had the effect of reducing the concentration of odor bearing substances in the white pans. One observation which is worthy of note is that in spite of generally low beet purities, sugar quality as measured by color and solution color seemed easier to maintain than usual.

Sugar Beet Male Sterility

R. K. OLDEMEYER[1]

Cytoplasmic male-sterility is the only practical, if not the only tool for enforcing hybridization on a large scale in sugar beets. Little if any genetic research has been done regarding this complex character in sugar beets since Owen's reports (6)[2] (7) in 1945 and 1950. Limited plantings of commercial sugar beet inbred x variety hybrids utilizing cytoplasmic male sterility have been made recently by some commercial companies. Corn breeders are now successfully using cytoplasmic male sterility for the commercial production of hybrids. Several sources of maize cytoplasm are available, (2) (5) and the one in use is capable of causing castration over a broad range of inbreds (8) (5). Very effective emasculation can be accomplished by the cytoplasm avialable in sorghums (9).

Sugar beet plants having genotypes, which with sterile cytoplasm, can be castrated, are rather rare in open-pollinated populations. This limits the proportion of inbred lines which can be made male sterile, and prevents the full exploitation of the male-sterile character unless a long and an intensive program is undertaken to transfer suitable genotypes to the necessary inbreds.

The purpose of this paper is to (a) report observations and results of experiments of the Great Western Sugar Company for the last two years regarding cytoplasmic male sterility and (b) point up the necessity for basic research regarding male sterility.

Indexing Variation Attributed to the Male-Sterile Parent

Intrasource variation overshadowed intersource variation as to castration of progeny from the same pollen parent when a Great Western source and a USDA (Owen) source of male sterility were compared. These results prompted a design of an experiment to determine the prevalence and degree of intrasource variation. The index of the progeny of one male-sterile plant was compared to the index of progeny from another male-sterile plant, when the pollen parent was the same.

The male-sterile population from which the test plants were chosen was heterogeneous, consisting of male-sterile plants found in the variety GW304 pollinated with the variety GW359, both varieties being very heterozygous and heterogeneous.

The use of male-sterile plants for indexing was confined to plants showing no visible signs of pollen in the anther, all other plants being rogued in the isolation plot. Pollination was accomplished by bag interchange, the pollen bags being brought from another isolation. One pollinator was used on as many as 10 different male-sterile plants. Pollinations were arranged so that one male-sterile parent had as many as 8 pollinations in common with other male-sterile plants.

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[2] Numbers in parentheses refer to literature cited.

Table 1.—Numbers and Percentage of Sterile, Intermediate and Fertile Progeny Produced by Various Male-Sterile Plants with Common Pollinators.

Male Parent	Sterile Plant Number											
	B623-1					vs	B623-2					
	Sterile		Inter.		Fertile		Sterile		Inter.		Fertile	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
51-412-1	18	78	5	22	0	0	17	53	15	47	0	0
-457-1	3	8	11	31	22	61	3	9	12	36	18	55
-457-3	8	29	15	54	5	17	8	20	22	55	10	25
-500-1	20	57	9	26	6	17	19	48	18	45	3	8
-517-3	3	23	9	69	1	8	8	23	22	69	10	8
-622-1	7	25	14	50	7	25	11	31	19	54	5	14
GWI-5-2	9	47	10	53	0	0	22	79	6	21	0	0
-7-1	29	81	7	19	0	0	17	65	7	27	2	8
Total	348		324		128		328		354		118	

Total $\chi^2 = 12.953$, P = .7-.5 for 14 d.f.

	B623-1					vs	B623-3					
51-412-1	18	78	5	22	0	0	14	44	18	56	0	0
-457-1	3	8	11	31	22	61	2	5	15	38	23	58
-457-3	8	29	15	54	5	17	15	38	16	40	9	23
-622-1	7	25	14	50	7	25	18	64	7	25	3	11
GWI-5-2	9	47	10	53	0	0	10	24	23	56	8	20
-6-2	1	3	23	57	16	40	1	2	14	35	25	63
-7-1	29	81	7	19	0	0	7	33	10	48	4	19
Total	271		286		143		210		298		194	

Total $\chi^2 = 32.543$, P = .01-.001 for 13 d.f.

	B623-2					vs	B623-3					
51-412-1	17	53	15	47	0	0	14	44	18	56	0	0
-457-1	3	9	12	36	18	55	2	5	15	38	23	58
-457-3	8	20	22	55	10	25	15	38	16	40	9	23
-622-1	11	31	19	54	5	14	18	64	7	25	3	11
GWI-5-2	22	79	6	21	0	0	10	24	23	56	8	20
-7-1	17	65	7	27	2	8	7	33	10	48	4	19
Total	257		240		102		208		263		131	

Total $\chi^2 = 34.085$, P <.001 for 11 d.f.

	B623-1					vs	B623-7					
51-412-1	18	78	5	22	0	0	15	44	15	44	4	12
-457-3	8	29	15	43	5	17	6	40	9	60	0	0
-622-1	7	25	14	50	7	25	2	6	27	77	6	17
GWI-6-2	1	3	23	57	16	40	1	2	5	12	34	85
-7-1	29	81	7	19	0	0	19	46	21	51	1	2
Total	216		202		82		138		244		116	

Total of $\chi^2 = 37.562$. P <.001 for 10 d.f.

Seed from crosses was planted at Salem, Oregon, in August, 1953. Visual readings were made on progenies in June, 1954. Progenies were divided into three classes: sterile, no pollen production; intermediate, trace to considerable pollen but no shedding; fertile, considerable to normal pollen which shed. Families (progenies from individual crosses) were indexed without the reader knowing the pedigree. From 10 to 40 offspring were included per family, with the average about 30.

The χ^2 method was used to determine the probability that any two male-sterile plants indexed a series of male plants alike. A number of male-sterile plants was compared in all possible combinations as to the proportion of sterile, intermediate and fertile progeny they produced. To make a comparison of any two male-sterile plants, a series of contingency tables was constructed, one for each male plant they had in common. The total χ^2 from the contingency tables measures the probability that the two male-sterile plants indexed the male plants the same. All but seven of the 30 comparisons had to be rejected as being different, P less than .05.

Contributing to the total χ^2 (Table 1) is the general trend for one male-sterile plant to produce more or less offspring of one classification than another male-sterile plant. This general trend is demonstrated by comparing the total percentage of sterile offspring (the most distinct class) produced by each male-sterile plant, comparisons limited to crosses with common pollinators. The ratios of steriles produced by one male-sterile plant as compared to the steriles produced by another are summarized in Table 2.

Table 2.—Ratios of the Total Percentages of Sterile Progeny for All Combinations of Male-sterile Parents. Only Steriles from Families Whose Inbred Pollinator Was Used on Both Male-sterile Plants Included in the Totals.

Male-Sterile Comparison	Sterile Ratio	Male-Sterile Comparison	Sterile Ratio
1 vs 2	1.06	3 vs 4	0.83
3	1.29	5	0.85
4	1.47	6	1.09
5	1.68	7	1.30
6	1.79	14	0.60
7	1.57	4 vs 5	1.71
14	2.38	6	0.92
2 vs 3	1.24	7	2.53
4	1.41	14	1.13
5	1.39	5 vs 6	1.42
6	1.19	7	1.22
7	1.24	14	0.66
14	2.16	6 vs 7	1.21

The ratio, 1.06, for 1 vs. 2 (Table 2) is obtained by dividing the total percentage in the sterile columns (Table 1) of B623-1, 348, by 328, the total percentage of steriles of B623-2. This indicates B623-1 produced a greater proportion of steriles than B623-2. Arranged in descending order in the production of steriles, the plants are 1, 2, 4, 14, 5, 3, 6, and 7. The only exception to this order is that 6 produced more than 4, but in all cases 4 produced more than 14, 5, 3, and 7; and 5 and 3 produced more than 6.

Another group of male-sterile plants was tested with plants from the variety A1183 (SL89) which was near "O" type. Only sterile and intermediate offspring resulted. Comparing the total of the sterile and intermediate offspring produced by common pollinators, as was done in the previous group of male-sterile plants, 14 of 21 comparisons had to be rejected, P less than .05. This again indicates little if any likeness exists between the plants as regards production of sterile progeny. The ratios of total percentages of sterile progeny produced by a set of common pollinators are tabulated in Table 3. Male-sterile plants in descending order of the percentage of male-sterile progeny produced, 59, 49, 103, 111, 108, 115, and 18, show no deviation from the order.

There are two possible causes for male-sterile plants producing different percentages of male-sterile progeny and falling into a continuous series as to percentage of sterile progeny when crossed to the same pollinators. The male-sterile plants might differ in (a) plasma genes, either in number or nature or (b) in content of minor modifying genes, genes not necessarily involved in differential interactions.

Testing for variations in plasma genes would involve backcrossing a homozygous inbred to several homozygous male-sterile plants suspected of having different plasma genes and observing the progeny during and at the end of a number of backcrosses. Differences in the male-sterile character of the progeny from the same inbred, but crossed to different male-sterile parents, could then be attributed to variation in plasma genes.

Also contributing to the total χ^2 (Table 1) is that variation which does not conform to the general trend for any one male-sterile plant to produce more or less sterile offspring. The general trend for B623-1 was to produce a greater percentage of steriles than B623-2, but the index of GWI-5-2 by these two plants was the opposite. Another example of the same variation is the indexing of plant 51-457-3 by plants B623-1 and B623-3. Such variation is evident, more or less, in all comparisons and is probably the result of genetic interaction in which the genes of a male plant complement the genes of one male-sterile plant differently than the genes of another male-sterile plant as regards their influence on pollen production.

The results of this study do not necessarily refute Owen's (6) postulation of 2 major factor pairs for the control of cytoplasmic male sterility in sugar beets. The major variations found between the indexes of different pollen plants on the same male-sterile plant may be due to differences in the two factor pairs. However, it is hardly conceivable that all the variation found in this group of male-sterile plants, as regards the fertility of their

progeny, can be attributed to some of the male-sterile tester plants being of a heterozygous Z genotype, xxZz, as reported by Owen (7). There is no doubt that the mechanisms involved in the conrol of cytoplasmic male sterility are more complicated than the two factor pair control postulated by Owen; the variability found in this study, whether caused by genes of minor action or by differences in cytoplasm, may have been present to cause the descrepancies noted by Owen (3).

Miscroscopic Pollen Examination

Pollen from a number of plants having male-sterile cytoplasm was stained with acetocarmine and examined with a microscope in order to have a basis upon which to coordinate visual readings with the actual fertilities. All stages of pollen abortion were present in a continuous series, from abortion in the tetrad stage, through normal pollen. The following classes were arbitrarily assigned:

Class	Microscopic description	Visual description
1.	Aborted, tetrad stage	No color in anther, sterile
2.	Aborted, without exine	No color in anther, sterile
3.	Aborted, little exine	Light yellow anther, sterile
4.	Aborted, well developed exine	Intermediate in fertility
5.	Less than 1% sound	Intermediate in fertility
6.	1-5% sound	Intermediate in fertility
7.	5-25% sound	Intermediate in fertility
8.	25-75% sound	Intermediate to fertile
9.	75-95% sound	Fertile
10.	95-100% sound	Fertile

Visual identification of the sterile group of classes, 1 through 3, is quite accurate. An experienced reader could differentiate the group of classes 4, 5, and 6, from class 7 with little overlapping. Fertile plants, classes 9 and 10, are quite difficult in some cases, to differentiate from classes 7 and 8. However, plants in the fertile classes generally have anthers which are packed with pollen. Only plants in classes 7 through 10 would reduce cross pollination significantly in a field of commercial hybrid seed, particularly if relatively self-sterile lines are used.

New and More Potent Sources of Cytoplasmic Male Sterility

A more potent source of cytoplasm, which would castrate plants with a wider range of genotypes than is possible with the present male-sterile cytoplasm, would make an inbred-hybrid breeding program much more flexible. Inasmuch as two new sources in wild *Beta* have been discovered by Great Western, new and as yet undiscovered sources probably exist.

A partially male-sterile *B. macrocarpa* Guss. (A1171) x sugar beet hybrid when backcrossed to sugar beet yielded a high percentage of male-sterile plants. A wild leaf beet introduced from Turkey, PEI 206411, was highly male sterile. Preliminary studies of these sources indicate they are very similar to, if not the same as the sources now available. In addition to these two new sources, there exists in Great Western stocks several additional sources which have not yet been studied.

Several instances of sterile cytoplasm existing in other species and races of genera have been reported for *Nicotiana* (4) and *Linum* (1). Caspari (3) reviewed the work of Michaelis who also found the phenomenon in *Epilobium*. In the above instances when the nuclear germ plasm of one species was placed, by backcrossing, into the cytoplasm of another, cytoplasmic male sterility resulted. A limited survey will be made by Great Western for male-sterile cytoplasm in other races of *B. vulgaris* and other species of *Beta*.

Summary

1. A group of sugar beet pollinator plants was used on a series of male-sterile plants so that the male-sterile plants had pollinators in common. An analysis of the progeny produced by different male-sterile parents with the same pollinator indicated genic interaction, influencing degree of fertility in male-sterile cytoplasm, exists between two parents and is not the same from one parental combination to another. Consistent differences in the proportion of sterile progeny existed between male-sterile parents over a range of common pollinators indicating either minor differences in genes not involved in interactions or variation in plasma genes.

2. Plants in a heterozygous population having male-sterile cytoplasm varied as to pollen abortion in a continuous series from abortion in the tetrad stage of development to normal pollen production. A classification based on microscopic examination of pollen is proposed.

3. Two new sources of male-sterile cytoplasm, one in *Beta macrocarpa* and one in a wild beet from Turkey, were discovered by Great Western. Preliminary investigation would indicate little difference between the action of the new sources and the sources already available.

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Nematode Cyst Hatch Rate as Influenced by Fractions of Beet Root Juice

H. M. Bauserman and R. F. Olson[1]

Introduction

The writers were interested in studying one phase of the relationship between sugar beets and the sugar beet nematode (*Heterodera schachtii*). The presence of certain plants near nematode cysts accelerates the rate of hatching of the eelworms, because of certain substances exuded from the plants into the soil. A relatively new method of separation, paper chromatography, was used to prepare fractions of sugar beet material, which fractions were tested for their influence on the hatching of the worms (cyst hatch rate). It was thought best to start with beet press juice because of its easy availability, in spite of the fact that the compounds in the soil may not be the same as or, in the same concentrations as the compounds found in the beet press juice. Moreover, there are certain disadvantages concerning root exudates, among others, variability resulting from various methods of collection, and the usual extreme dilution of such exudates.

Baunacke (1)[2], and Wood and Serro (2) have shown that the cyst hatch rate of the nematode, *Heterodera schactii* is greatly affected by the presence of certain stimuli. Rietberg (3) mentions that the cyst hatch rate is strongly stimulated by root exudates of certain tested plants, which were mostly host plants. Hijner and den Ouden (4) have studied the hatching of larvae from the cyst and also the substances which influence the hatching. They developed a more or less standardized method for the production of root exudates, and analyses were started on the root exudates. They also state that large numbers of substances were tested for their effect on hatching. Wood and Serro (2) have identified galactinol in the root exudate of sugar beets. This laboratory determined galactinol in the press juice from sugar beets grown in a field severely infested with nematode. Finkner and Swink (5) found a relation between the tons per acre yield and low galactinol.

In certain field trials, some beets seemed to display varying degrees of resistance to nematode attack. By chromatographing the beet press juice from selected beets, all substances including those that either enhance or inhibit the cyst hatch rate, were more or less separated from one another, and were studied as individuals or fractions. Separating compounds into fractions reduces the synergistic effects. To confirm the observed influence of fractionated beet press juice on cyst hatch rate, various concentrations of pure substances were similarly tested.

This entire series of tests was preliminary in nature and this paper is a report on the progress made to date.

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[1] Head Research Chemist and Research Chemist respectively, American Crystal Sugar Company, Rocky Ford, Colorado.

[2] Numbers in parentheses refer to literature cited.

Materials and Methods

Seven varieties of beets that differed in tons per acre yield and percent galactinol in the beets were rasped and juice was pressed from the pulp. Table 1 gives three characteristics of the varieties.

Table 1.—Yield Data of Varieties Used in Experiments.

Varieties	Tons Per Acre	Percent Galactinol	Percent Sugar
7-1	4.22	0.078	17.6
4-1	3.70	0.114	16.7
39-1	3.70	0.114	16.7
9-1	3.14	0.110	15.8
8-1	2.53	0.146	15.4
40-1	1.43	0.242	15.7
30-1	1.23	0.244	12.8
23-1	0.83	0.308	11.9
46-1	0.83	0.308	11.9

Fifteen microliters of the expressed juice, after two fold dilution, were spotted on a one-inch wide strip of Whatman No. 1 chromatography paper. A solvent was used, which consisted of seventy parts iso-propyl alcohol, ten parts n-butanol, twenty-five parts benzene and twenty-five parts water. The various constituents of the beet press juice were separated into fractions by allowing this solvent to flow down the strip for a distance of approximately twenty-one inches. Following the irrigation period the paper was dried and cut into thirteen sections. The first section contained the original spot and the last section contained the solvent front. Similar strips were spotted with amino acid, galactinol, inositol, raffinose, sucrose and saponin in order to locate the position of the constituents in the various fractions of the beet press juice chromatograms. The compounds, which compose the greatest percent of dry substance in the beet and which were determined by paper chromatography, are as follows:

Fraction	Constituents in Fraction
1	Original spot
2	Galactinol, raffinose and ninhydrin reactant (x)
3	Raffinose, inositol, kestose, glutamic acid, asparagin and aspartic acid
4	Sucrose and glutamine
5	Sucrose, dextrose, saponin, glycine and gamma amino butyric acid
6	Fructose and alanine
7	Valine
8	Unknown
9	Isoleucine
10	Ninhydrin reactant (y) and fats
11	Ninhydrin reactant (y) and fats
12	Unknown
13	Solvent front

Each section was placed in one milliliter of distilled water. A few drops of the resulting solution were placed in a cell containing three nematode cysts. Each cell was observed through a microscope and the number of eelworms hatched were counted every twenty-four hours.

With the exception of inositol, galactinol and glutamic acid, the concentration of the individual chemical substances tested is near that of the concentration in the chromatographed beet press juice. The three chemicals mentioned have more or less the concentrations found in root diffusates as prepared by Wood and Serro (2).

Cells 5 mm. in diameter were made in a paraffin layer about 3 to 4 mm. deep in a petri dish, according to the method of Wood and Serro (2). To retard the evaporation of the solutions from the cells a moist blotter was inserted in the top half of petri dishes. Each experiment was continued for a period of approximately thirty days. The experimental results are found in Tables 2, 3 and 4.

Nematode cysts were obtained for these tests by taking beets from a field severely infested with nematode and bringing the roots with adhering soil and cysts to the laboratory. Some soil was removed from the roots to expose the cysts, which were then removed from the small rootlets. This method of obtaining cysts may differ from the soil screening method in the amount of force necessary to disengage cysts from rootlets or soil. Cysts obtained by different methods may react differently. Cyst variability or other factors caused variable hatch rate to such an extent that study of replications indicates our results were not highly significant.

Table 2.—Number of Eelworms Hatched per cyst per Fraction in Solutions of Chromatographed Beet Press Juice. Average of Three Replications.

Fraction Number	Eelworms per Cyst					Over-all Fraction Average
	Variety 7-1	Variety 39-1	Variety 46-1	Variety 30-1	Variety 23-1	
1	15.4	8.6	5.0	0.0	2.0	6.2
2	25.5	6.0	0.0	10.2	5.6	10.1
3	6.8	0.0	5.5	5.9	13.8	6.4
4	22.0	9.6	4.8	4.1	4.0	9.1
5	0.1	1.5	3.3	0.3	15.2	3.6
6	13.3	3.2	11.6	0.1	6.1	6.9
7	0.4	6.8	0.8	0.1	17.9	5.0
8	9.4	0.8	5.6	0.0	4.8	4.1
9	2.9	20.4	0.8	0.7	1.8	5.3
10	8.4	1.1	13.3	8.7	4.9	7.3
11	4.8	9.8	4.4	5.8	11.0	7.2
12	2.8	22.6	8.4	2.2	1.4	7.3
13	3.8	6.7	0.2	0.0	2.3	2.5
Distilled H_2O	4.7	5.3	3.5	2.3	8.3	4.8

Table 3.—Number of Eelworms Hatched per Cyst per Fraction in Solutions of Chromatographed Beet Press Juice. Average of Two Replications.

Fraction Number	Eelworms per Cyst						Over-all Fraction Average
	Variety 7-1	Variety 4-1	Variety 9-1	Variety 8-1	Variety 40-1	Variety 23-1	
1	1.7	6.2	0.0	7.0	2.4	9.2	4.4
2	1.3	0.0	2.0	10.0	3.5	1.0	3.0
3	0.0	1.0	0.2	2.2	1.2	0.2	0.8
4	11.5	0.2	6.0	3.4	7.9	2.2	4.6
5	0.2	0.4	0.4	0.8	4.0	0.0	0.9
6	0.0	1.0	20.0	0.0	2.0	5.0	4.7
7	2.2	0.7	2.0	0.0	0.0	1.5	1.1
8	1.2	3.5	5.2	0.0	0.0	2.2	1.9
9	1.0	1.7	4.0	0.2	0.0	4.5	1.9
10	0.0	5.2	0.0	0.2	2.5	5.7	2.3
11	0.0	0.4	12.2	11.9	0.4	4.5	4.9
12	0.4	0.0	4.7	4.9	5.0	1.0	2.6
13	1.3	0.0	2.8	0.4	6.4	6.7	2.9
Distilled H_2O	2.3	0.0	2.0	2.2	3.5	2.0	2.0

Table 4.—Number of Eelworms per Cyst in Solutions of Twelve Chemicals and Distilled water.

Chemical Solution	Concentration p.p.m.	Eelworms per Cyst		
		Replication a	Replication b	Average a + b
Inositol	50-150	0.33	0.56	0.44
Galactinol	2-7	5.33	4.89	5.11
Glutamic acid	2-7	6.77	6.67	6.72
Glycine	2-6	7.33	5.33	6.33
Alanine	2-6	2.33	2.78	2.56
Valine	2-6	2.22	7.11	4.67
Isoleucine	2-6	6.89	0.44	3.67
Glutamine	10-20	6.22	4.00	5.11
GABA	2-6	1.78	0.44	1.11
Aspartic acid	2-6	3.56	1.33	2.45
Sucrose	500-1500	16.11	3.33	9.72
Saponin	8-12	6.00	1.56	3.78
Distilled water — Average of twelve tests				4.42

Conclusions

Certain fractions of the chromatographed beet press juice appear to enhance or inhibit the cyst hatch rate when compared with the hatch rate in distilled water. Figure 1, a bar graph, shows the average of the adjusted total number of eelworms hatched per cyst per fraction for all varieties and replications at the end of thirty days. The fractions that may inhibit are 5 and 7. The fractions that may enhance the hatch rate are 1, 2, 4, 6 and 11.

Table 5.—Comparison of Fraction Enhancing or Inhibiting Power with Individual Compound Reactions.

Fraction No.	Fraction Reaction	Known Compounds Present	Individual Compound Reaction
2	Enhance	Galactinol	Enhance
4	Enhance	Sucrose	Enhance
		Glutamine	Enhance
6	Enhance	Fructose	Enhance
		Alanine	Enhibit
11	Enhance	Ninhydrin Reactant	—
		Fats	—
3	Inhibit	Inositol	Inhibit
		Aspartic Acid	Inhibit
		Glutamic Acid	Enhance
		Kestose	—
		Raffinose	—
		Asparagine	—
5	Inhibit	Sucrose	Enhance
		Saponin	Inhibit
		Dextrose	Enhance
		Glycine	Enhance
		GABA	Inhibit
7	Inhibit	Valine	Enhance

The enhancing or inhibiting power of the fractions may be correlated to certain chemicals present in the fractions, Table 5. From the experiment conducted with pure substances, the following tentative conclusions were drawn:

1. Inositol, alanine, isoleucine, gamma amino butyric acid (GABA), aspartic acid and saponin in general tend to inhibit the hatching of eelworms from cysts when compared with distilled water.

2. Galactinol, glutamic acid, glycine, valine, glutamine and sucrose appear to enhance the emergence of eelworms from cysts when compared with distilled water.

Distilled water, which was used as a comparison, exhibited a variable pattern of cyst hatch rates. We confirm Rietberg's (3) observation that cyst hatch can occur in the absence of a stimulant, other than water.

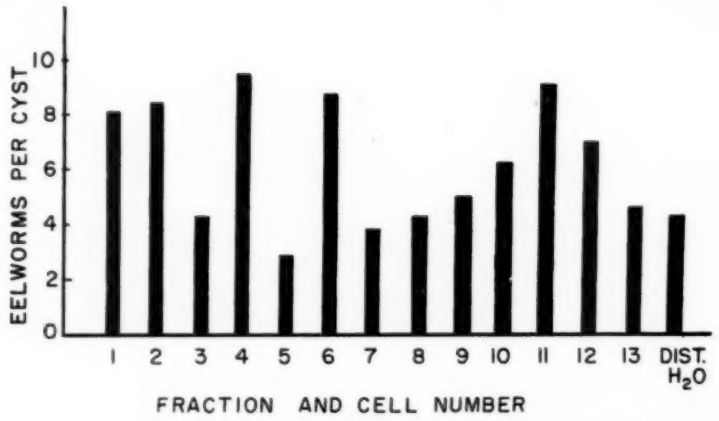

Figure 1.—Average of the adjusted total number of eelworms hatched per cyst per fraction for all varieties and replications at end of thirty days.

Saponin appears to have an abnormal effect on the eelworms that have hatched from the cysts. After a period of approximately three days the eelworms become immobile and unbending within the cells.

Because certain groups of beets reacted differently while growing in the field, it was thought possible that certain fractions would show differences between varieties. Even though there were great differences in the hatching rates, too few tests were run to obtain highly significant results.

Acknowledgment

The authors are indebted to J. F. Swink and R. E. Finkner for the beet roots and yield data used in these tests.

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Methods for Count of Microorganisms in Liquid Sugar

H. E. HALDEN[1]

During the past 25 years, and particularly since 1945, there has been a steady increase in the manufacture and use of liquid sugars. The demand has reached such proportions that liquid sugars now constitute a considerable portion of the total sugar production. With the increase in production of liquid sugars certain problems have developed. In the early days the liquid sugars were manufactured and consumed within a relatively short time, while now, with increased shipping distances and the greater demand, the time between manufacture and use has increased considerably.

Due to the nature of liquid sugar products they are far more susceptible to deterioration caused by spoilage organisms than dry sugars. While perfectly sterile products may be manufactured, they may be exposed to spoilage organisms in plant storage, during shipment and in the customer's tanks. The most scrupulous cleanliness is naturally a requisite for all liquid sugar equipment. In order to check the effectiveness of the cleaning procedure of equipment and preventative measures taken against contamination, a well planned microbiological control becomes the most effective weapon.

The organisms with which we are the most concerned are:

1. Mesophilic aerobic bacteria
2. Yeasts
3. Molds

All of these organisms may be airborne and while the optimum growth temperatures range from 28° to 40° C., some may be capable of resisting much higher temperatures for short periods.

Of the three groups of organisms the mesophilic aerobic bacteria are of least importance. They are rarely, if ever, responsible for deteriorative changes in sugars. Their presence, however, is an indication of the amount of airborne contamination occurring during storage and transportation of the liquid sugars. Yeasts and molds multiply rapidly when growth conditions are favorable and may cause serious damage to liquid sugars. The presence of these organisms, even in very small numbers, may eventually be a serious threat to the keeping quality of liquid sugars under unfavorable storage condiions.

The problems involved in a quantitative determination of a very low count do not appear to be generally understood. The difficulties in obtaining a small representative sample can readily be appreciated when we consider the following.

The organisms present in liquid sugar are not evenly distributed. With a very small population the chances for getting a representative count on a small sample are very remote, in particular from a tank which has been standing for some time. It has been demonstrated by Owen (1) that

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[2] Numbers in parentheses refer to literature cited.

yeast colonies have a tendency to migrate to the surface and that consequently, the main body of the syrup may show a low organism count while the surface would have a higher concentration than the average for the whole tank.

The above facts should be kept in mind when obtaining a sample of liquid sugars for microbiological examination. One sample from a freshly prepared syrup may be sufficient for the evaluation of its organism content. It would, however, be necessary to take samples from different levels of a tank which has been standing for some time. Thorough agitation of the syrup in the tank before sampling would make it possible to take less samples and expect a good evaluation. No specific instructions can be given to cover all cases, since each one must be considered by itself.

When the final sample is made up from one or more subsamples it must, with reasonable assurance, be representative of the whole tank of syrup. The final sample should not be too small, preferably at least one pint.

Additional problems come up when a portion is removed from the sample for analyses. A single portion of a few grams cannot represent the whole sample if the population is low. This situation is far more pronounced than that of the initial sampling of the whole tank.

At Spreckels Sugar Company's microbiological laboratory, five portions of the sample, each representing 20 grams of dry sugar, are weighed into five 250 ml. Erlenmeyer flasks.

Each flask is made up to 100 ml. with sterile water making sugar solutions of 20 percent strength W/V. From each of these flasks, five dextrose-tryptone agar plates for bacteria, and five wort agar plates for yeasts and molds are prepared. Two ml. of sugar solution is used for each plate. The original sample will be represented, therefore, by 25 plates for the count of bacteria, and 25 plates for the count of yeasts and molds. Twenty-five plates with 0.4 grams sugar on each represent 10 grams of dry sugar.

In a count of microorganisms we are dealing with a discontinuous distribution. With a limited number of microorganisms, 100 in 100 ml., we would find in 100 samples of one ml. each, a number containing no organisms and a number with one or more.

Among discontinuous distributions the Poisson series is probably of first importance. It has sometimes been called the law of small numbers.

If the probability of an event is exceedingly small, the number of occurrences of the event in a sufficiently large number of independent cases will be distributed in the Poisson series.

Tables have been prepared by F. G. Eis (2) to show the accuracy that may be expected in plate counts using 0.4 gram of sugar per plate. The tables give plate count per 10 grams, actual for 25 plates and calculated to 10 grams for other numbers. All values are based on the Poisson series and a technique of sampling and dilution affording a random distribution of organisms.

Table 1.—Percentage of the Time a Zero Count May Be Expected at Various Actual Average Organism Levels.

Actual Product Average Org./10 gr.	Number of Plates Counted (.4 gr. sugar, each)							
	1	2	5	10	25	50	100	200
1	96	92	82	67	37	14	1.8	0.03
5	82	67	37	14	0.7	.004		
10	67	45	14	1.8	.004			
20	45	20	1.8	0.03				
100	1.8	0.03						
200	0.03							

Example: Using 25 plates, each containing 0.4 grams dry sugar, a count of zero may be expected 37 percent of the time when there is an average of one organism per 10 grams of sugar. With an actual population of 10 organisms per 10 grams of sugar the chances for getting a zero count are practically nil.

Table 2.—Expected Count /10g. at Various Actual Organism Levels (Not more than 5 percent under minimum or 5 percent over maximum value listed).

Actual Product Average Org./10 gr.	Number of Plates Counted (.4 grams sugar, each)								
	1	2	5	10	25	50	100	200	
1	0	0	0	0	0	0	0	1	Min.
	25	25	10	7	4	3	2	2	Max.
5	0	0	0	0	2	3	3	4	Min.
	50	38	20	15	10	8	7	6	Max.
10	0	0	0	3	5	6	7	8	Min.
	75	38	30	23	16	15	13	12	Max.
20	0	0	5	10	13	15	17	Min.	
	75	63	45	35	29	26	24	Max.	
50	0	13	25	33	39	42	Min.		
	150	113	100	73	63	59	Max.		
100	25	50	65	75	84	Min.			
	225	175	145	130	118	Max.			
200	100	125	150	165	Min.				
	350	300	260	240	Max.				

Example: Using 25 plates, each containing 0.4 grams dry sugar, a count of from 5 to 16 may be expected in 90 percent of trials when the actual product average is 10 per 10 grams. In 5 percent of trials the count may be expected to be less than 5 and in 5 percent of the trials the count may be expected to be greater than 16.

Table 3.—Expected Range of Average Number of Organism Per 10 gram at Various Plate Counts (Not more than 5 percent under minimum or 5 percent over maximum value listed).

Count Per 10 g.	Number of Plates Counted (.4 grams sugar, each)							
	1	2	5	10	25	50	100	200
0	0	0	0	0	0	0	0	0 Min.
	73	36	15	7	3	1	1	1 Max.
1					1	1	1	1 Min.
					5	3	2	2 Max.
3				1	1	1	2	2 Min.
				12	8	6	5	4 Max.
5			1	1	1	2	3	4 Min.
			24	16	11	8	7	7 Max.
10			1	2	5	6	8	8 Min.
			31	23	16	13	13	12 Max.
25	1	1	7	12	17	20 Min.		
	118	78	53	40	34	32 Max.		
50	2	11	24	32	39 Min.			
	155	114	80	73	63 Max.			
100	23	41	63	75 Min.				
	228	180	145	130 Max.				
200	83	116	150	163 Min.				
	328	288	260	240 Max.				
400	233	288	325 Min.					
	575	501	480 Max.					

Example: In a sample, in which the organism count has been determined to be 25 per 10 grams, the actual product average may have been from 17 to 34 per 10 grams of sugar in 90 percent of trials, when 25 plates are used. In 5 percent of trials the product average may have been less than 17 and in 5 percent of trials more than 34.

The statistical treatment shows that 25 plates, containing 10 grams of sugar, give a fair approximation of the average number of organisms present per 10 grams of sugar. While 25 plates probably represent the practical limit when several samples are to be tested, it is also evident that more plates might be preferable for greater accuracy when the organism population is very low. It is not practical nor advisable to increase the sugar content per plate without making an adjustment in the nutritives medium. Such adjustment would in itself require considerable time and trials.

The statistical treatment has shown the accuracy with which low counts can be determined by plating techniques. Maximum limits placed on a product, in a range where methods of counting are not accurate, may be subject to question as to the actual necessity for such limits. If such low limits are an actual necessity, then a different approach to the quantitative determination of organism levels is necessary.

A new approach to the estimation of the population of microorganisms in liquids and gases has been proposed by manufacturers of a new filter membrane. This membrane is stated to be composed of cellulose esters with a porosity of 80-85 percent, the porosity being defined as the ratio of voids or pore volume to the volume of structural elements. Two basic types are available, one with a pore size of 0.45 micron, recommended for hydrosol assay and one with a pore size of 0.8 micron, recommended for aerosol assay.

The filter membrane may be sterilized or may be obtained in a sterile package. The sample of liquid or gas is passed through the membrane placed in a suitable holder. The membrane is then incubated in a Petri dish at a specific temperature on a pad containing a selective medium. The organisms, favored by the selective medium, develop in a much shorter time than that required in conventional methods and may be stained and counted directly from the pad.

Membrane filters have been used for the direct count of bacteria since 1933 or earlier. During the last five years, an increasing use has been found for molecular membrane filters for the examination of water and sewage and in the dairy industry.

Two bottling companies, the Pepsi Cola Bottling Company and Belfast Beverages, Incorporated of San Francisco and Oakland are currently using the membrane filter method in conjunction with other microbiological methods.

To our knowledge membrane methods have not otherwise been widely used on sugar products. Trials conducted at the Central Quality Control Laboratory of the Spreckels Sugar Company at Woodland, California, indicate that the filter membrane may permit the use of larger samples and an increase in the number of samples which can be handled by the microbiological laboratory. Due to the low flow resistance of the membrane filter it is possible to filter 150 grams of liquid sugar (100 grams of dry sugar), diluted to about 20 percent sugar, in about 3 to 4 minutes.

The filter holder, which is shown in Figure 1, is manufactured by the A. G. Chemical Company, of Pasadena, California. It consists of two sections. The upper is made of stainless steel with an anodized aluminum base which locks onto the lower section. The lower section, also made of stainless steel, contains a porous carbon filter disk on which the membrane filter is placed. Before use the filter assembly may be sterilized by autoclaving, dry heat or flaming. However, the most convenient method is probably sterilization by the incomplete combustion of methyl alcohol.

For this method a special sterilizing base is used. This base contains a porous carbon disk which is saturated with methyl alcohol. The alcohol is ignited and the upper section of the holder assembly is placed on the base. After a few seconds the lower section of the assembly is placed in an inverted position over the funnel. The formaldehyde, formed by the incomplete combustion of the methyl alcohol, is sufficient to completely sterilize the assembly in 15 minutes.

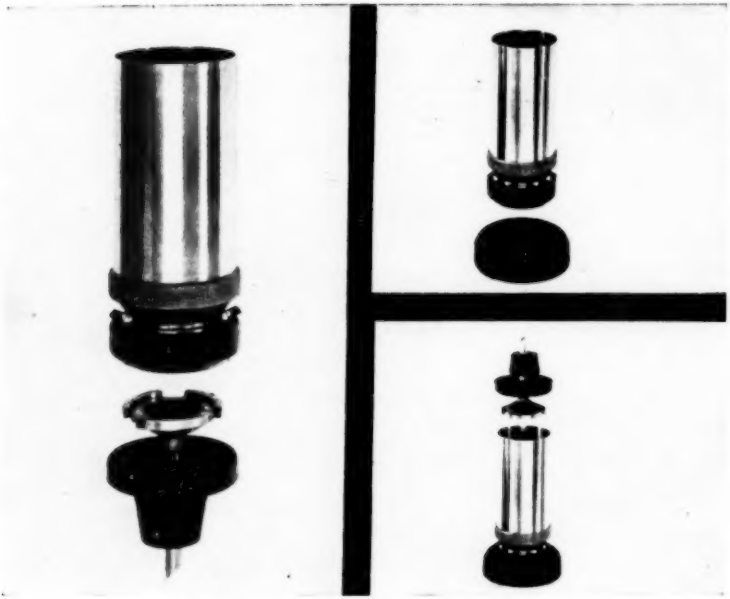

Figure 1.—Filter holder assembly.

The membrane filters are supplied with a protective paper cover and an absorbent pad which may be obtained with or without a nutritive medium. The absorbent pad is placed in a 60 mm. Petri dish and saturated with 2.3 ml. of sterile water (for prepared pads) or the same amount of a nutritive medium. The protective pad is not removed from the filter until it is placed in the holder. After filtration the filter is placed on the absorbent pad. The dish is then covered and incubated in an inverted position. To prevent loss of moisture from evaporation the dish may be sealed with a plastic film or tightly wrapped in aluminum foil. The incubation period is 24 to 36 hours at 30° C. for yeast and 12 to 18 hours at 37° C. for aerobes.

The organisms may be stained before viewing them microscopically.

If it is desired to express the count of organisms on the basis of 10 grams of dry sugar, the total count of organisms on the filter is divided by 10 if a total of 100 grams dry sugar (150 grams liquid sugar) has been used.

Figure 2 shows a comparison of the accuracy which may be expected by using 100 grams of sugar in the membrane filter method instead of 10 grams in the 25 plate method.

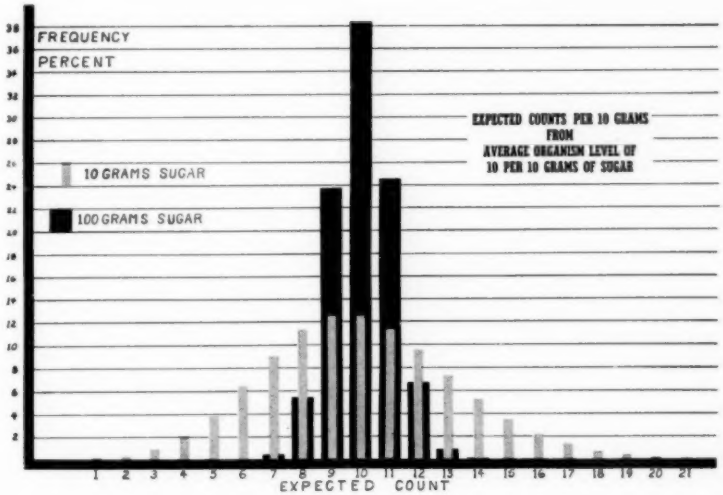
FREQUENCY
PERCENT
10 GRAMS SUGAR
100 GRAMS SUGAR
EXPECTED COUNTS PER 10 GRAMS FROM AVERAGE ORGANISM LEVEL OF 10 PER 10 GRAMS OF SUGAR
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21
EXPECTED COUNT

Figure 2.—Expected counts per 10 grams from average organism level of 10 per 10 grams of sugar.

Summary and Conclusions

Liquid sugar products are more susceptible to deterioration caused by spoilage organisms than dry sugar. Accurate quantitative determination of very small numbers present is considered to be important for at least two reasons:

1. Accurate counts are necessary for a well planned microbiological control program during storage and shipment.

2. Accurate counts of small numbers of organisms are necessary so that specific limits may be established for consumers acceptance. Limits based on inaccurate determinations may work hardships on both the producer and the consumer.

A statistical evaluation of the plating technique has shown that an excessive number of plates are required for accurate counts at low average organism levels. A new approach has been proposed by manufacturers of a new filter membrane. This new approach may result in a better control program and allow an accurate estimation of the number of organisms in a sample.

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Growth and Nutrient Uptake of Sugar Beets in the Imperial Valley, California[1]

A. J. MacKenzie, K. R. Stockinger, and B. A. Krantz[2]

A thorough knowledge of the growth pattern and nutrient requirements of sugar beets is essential to a better understanding of the fertility requirements for the maximum production of sugar. To plan an efficient fertilizer program, an understanding of the growth and nutrient-uptake curves is necessary.

During the 1953-54 season, a study was conducted to collect such information for sugar beets growing in the Imperial Valley area of California. The nitrogen uptake and dry-matter production throughout the season was determined as well as the total phosphorus and potassium uptake by the plants at the end of the season.

Methods and Procedure

In the 1953-54 season sugar beets were grown in an experiment with four rates of nitrogen application each, on four levels of available soil moisture on the stratified phase of Holtville silty clay at the Southwestern Irrigation Field Station, Brawley, California. Seven of these treatments were selected for detailed study of dry-matter production and mineral uptake. A description of the nitrogen and soil-moisture treatments and the resulting yield of beets and sugar and sugar percentage are given in Table 1.

Table 1.—Description of Treatments Selected, Number of Irrigations, and Their Effects on Yield of Beets and Sugar and Sugar Percentage.

Nitrogen	Soil-Moisture Tension Prior to Irrigation	Irrigations	Beets	Sugar	Sugar
Lbs. N/A	Atmospheres	Number	Tons/A.	Tons/A.	%
0	0.3	17	21.5	3.31	14.6
80	0.3	17	24.3	3.88	16.0
160	0.3	22	27.6	4.32	15.6
320	0.3	23	29.0	4.15	14.4
320	0.7	17	30.7	4.48	14.6
320	2.0	12	27.2	3.96	14.6
320	6.0	10	26.6	3.72	14.0
L.S.D. (.05)			5.2	0.88	NS
L.S.D. (01)			6.9	1.16	NS

[1] Contribution of the Western Section of Soil and Water Management, Soil and Water Conservation Research Branch, Agricultural Research Service, U. S. Department of Agriculture.

[2] Chemist and Soil Physicist, Southwestern Irrigation Field Station, Brawley, California, and Area Supervsor, Billings, Montana, respectively.

The soil-moisture levels were controlled by irrigation whenever the tensiometers or gypsum blocks installed in the plots at the eight-inch depth indicated the desired tension had been reached. The nitrogen, where applied, was sidedressed as follows: 32 pounds per acre at planting, one-half of the remaining nitrogen at thinning and the last one-half of the remaining nitrogen when the weather warmed up in January. All treatments received 40 pounds of P_2O_5 per acre banded three-inches to one side and three-inches below the seed at planting. The beets (US 56/2) were planted in single row 30-inch beds on October 1 and harvested in early July.

From the seven treatments in the experiment selected for the growth and nutrient-uptake study, samples of the beets were taken periodically at 80, 120, and 150 days after planting and at 20-day intervals thereafter until the end of the growing season. A six-foot segment was taken at random from one of two rows on either side of the center row from each of the four replicates for each treatment. The tops and roots were weighed, subsampled, dried in a forced draft oven at 65° C., reweighed for calculation of dry-matter content and then ground for chemical analysis.

The sugar beet tops and roots were analyzed for total nitrogen at all sampling dates. Total phosphorus and potassium were determined only at the last three sampling dates. Nitrogen was determined by the Gunning procedure (1)[2]. Samples for phosphorus and potassium analysis were wet ashed by digesting in nitric and perchloric acids. Phosphorus was determined colorimetrically using the vanado-molybdate method (2) and potassium was determined with a Beckman D.U. spectrophotometer equipped with the oxygen-acetylene flame attachment.

Results and Discussions

In the over-all experiment, as partially indicated in Table 1, soil moisture level had no significant effect on yield of beets or sugar, or on the percent sugar. The trend in yield, however, was in the direction of higher yields with the "wetter" treatments. Nitrogen fertilizer increased yield of beets and sugar. The yields were still increasing at nitrogen levels of 320 pounds per acre. There was a slight depression in the sugar percentage at the high-nitrogen rate but the differences were not significant. There was no significant interaction between nitrogen and moisture. The yield increases due to the different rates of nitrogen were similar at all levels of soil moisture. The top yield per acre was 4.48 tons of sugar and 30.7 tons of beets obtained with the application of 320 pounds of nitrogen per acre and 17 irrigations.

Dry-Matter Production

The total dry-matter production for the seven treatments is shown in Figure 1. Application of nitrogen to the 0.3 atm. soil-moisture treatment increased the dry-matter yield of both tops and roots. The 320 pounds of nitrogen per acre rate produced 12.1 tons of dry matter per acre, an increase of 4.2 tons per acre over the no-nitrogen treatment. The treatments in which the soil-moisture tension was allowed to reach higher levels prior to irrigation showed a trend toward lower yields of dry matter.

[2] Numbers in parentheses refer to literature cited.

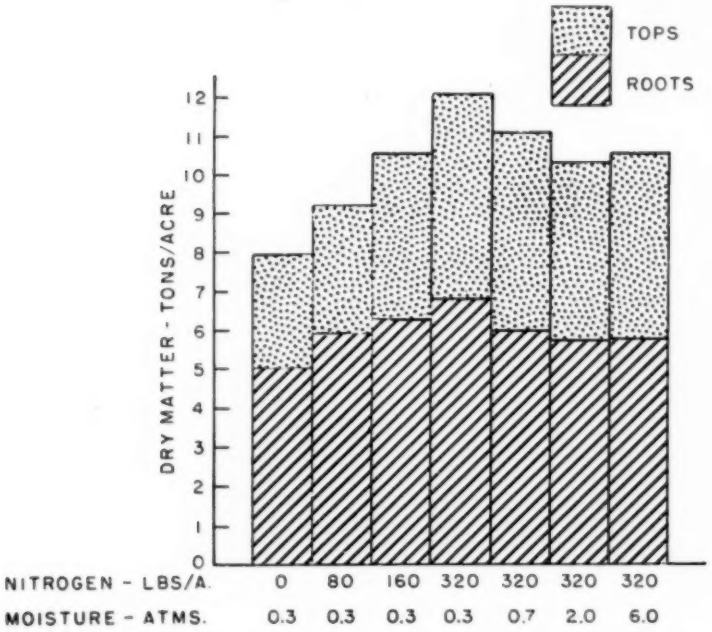

Figure 1.—The production of dry matter by sugar beets under various nitrogen and soil-moisture treatments, Imperial Valley, California, 1954.

Slightly less than half of the dry matter was produced as tops (36 to 46 percent). Application of nitrogen increased the top-root ratio while the soil-moisture variables did not affect the ratio. For the treatments producing the highest yields of beets (26 to 30 tons), an average of 4.8 tons per acre of dry tops was obtained.

The accumulative dry-matter production curve for beets receiving 320 pounds of nitrogen is shown in Figure 2. The growth curve for the whole plant as well as for the tops and roots is given over the 270 day growing period from October to July. During the first 90 days of growth—until early January—the rate of growth was slow. During that time only 12.5 percent of the total dry matter was produced. Of this amount approximately one-third was roots and two-thirds was tops.

As temperatures increased about the middle of January the rate of growth increased rapidly. Dry-matter production of both tops and roots increased for the next 125 days or until the early part of May. Then the growth of the tops reached its maximum and no further increase in top growth was obtained. During the last two months of the growing season, when high daily temperatures were common, the tops of the beets began to

dry and disintegrate. This effect was most noticeable with older leaves and petioles where the plants were subjected to the dryer soil-moisture treatments. Because of this, it was difficult to recover all of the tops during sampling and lower dry matter weights for tops were obtained at the last two sampling dates (see Figure 2). The roots, however, continued to grow and reached their maximum dry weight at harvest. After the tops had reached their maximum weight, continued root growth until harvest accounted for approximately 28 percent of the total root dry matter produced which was equivalent to seven tons increase in yield of beets.

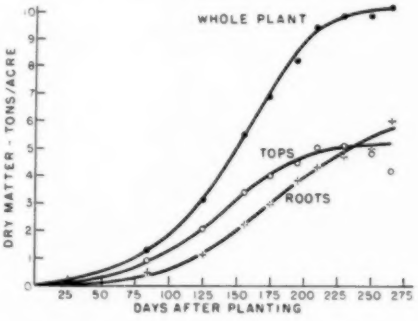

Figure 2.—The accumulative dry-matter production curve of sugar beets receiving 320 lbs. N per acre, Imperial Valley, California, 1954.

The shapes of the dry-matter production curves for beets receiving less nitrogen fertilizer were similar to those in Figure 2, however, the rates of growth during the periods of maximum dry-matter production were much less.

Nitrogen Uptake

The effect of treatments on the uptake of nitrogen (Figure 3) was similar to the effect on dry-matter production. The highest total nitrogen uptake, 429 lbs. per acre, was obtained from the same treatment which resulted in maximum dry-matter production (320 pounds of nitrogen per acre and 0.3 atm. soil moisture). Applications of less than 320 pounds of nitrogen per acre reduced significantly the uptake of nitrogen. Beets which received no nitrogen were able to derive 236 pounds of nitrogen per acre from the soil. Thus, the beets receiving 320 pounds of nitrogen contained 193 pounds more nitrogen than those in the no nitrogen treatment, which is an uptake of approximately 60 percent of the applied nitrogen. Changes in soil moisture had little effect on the uptake of nitrogen, although there was a trend toward lower nitrogen uptake with increasing soil-moisture tension prior to irrigation.

At the end of the season a greater amount of nitrogen was contained in the tops than in the roots. When fertilized with 320 pounds of nitrogen per acre, the tops contained 60 percent of the total nitrogen taken up or approximately 240 pounds of nitrogen per acre. Removal of sugar beet tops from the land could be expected then to reduce the potential nitrogen fertility of the soil by that amount. Removal of the roots alone would take about 160 pounds of nitrogen per acre from the soil.

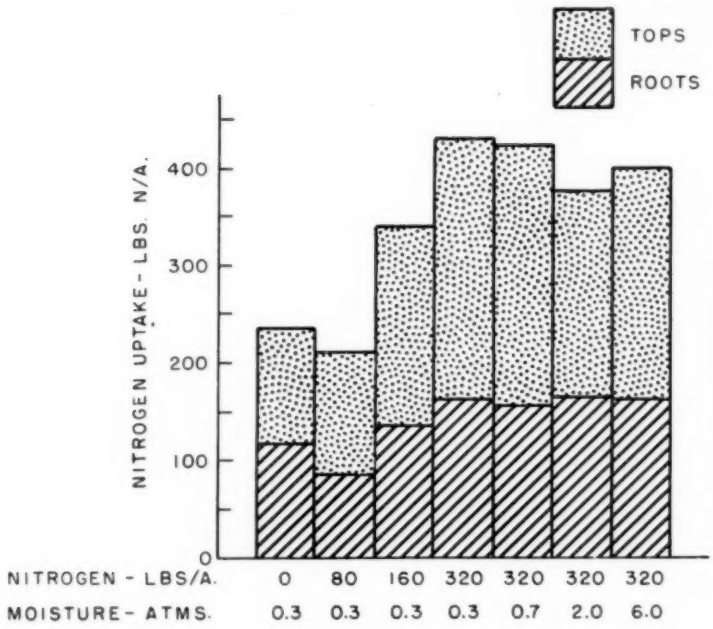

Figure 3.—The uptake of nitrogen by sugar beets under various nitrogen and soil-moisture treatments, Imperial Valley, California, 1954.

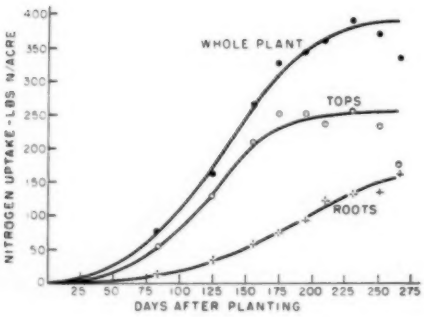

Figure 4.—The accumulative nitrogen-uptake curve of sugar beets receiving 320 lbs. N per acre, Imperial Valley, California, 1954.

The accumulative nitrogen-uptake for sugar beets receiving 320 pounds of nitrogen per acre is shown in Figure 4. The shape of the nitrogen-uptake curves are very similar to those of dry-matter production (Figure 2). Maximum rate of growth was accompanied by maximum rate of nitrogen uptake. By January, after the first 90 days of growth, the sugar beets had taken up

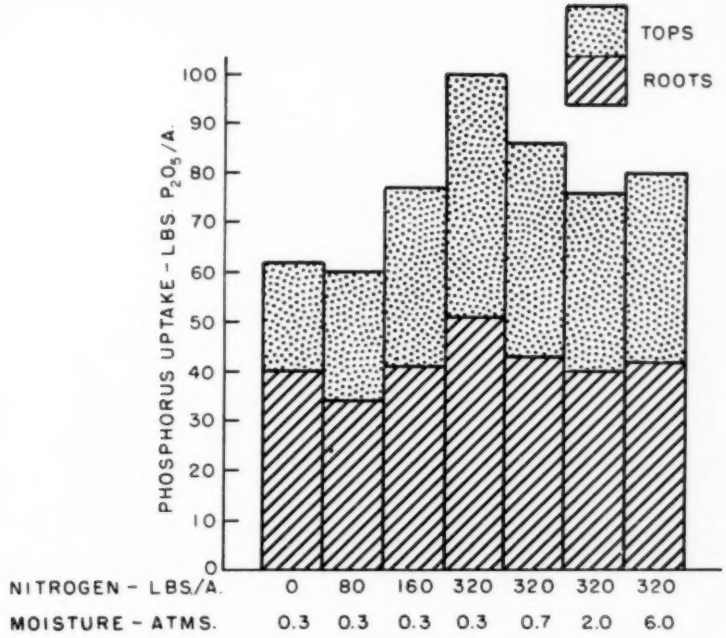

Figure 5.—The uptake of phosphorus by sugar beets under various nitrogen and soil-moisture treatments, Imperial Valley, California, 1954.

only 20 percent of its nitrogen or 75 pounds of nitrogen per acre. During the next 125 days of vigorous growth or until early May, the plants used 285 pounds of nitrogen per acre. Nitrogen uptake for the tops reached its maximum in late April (175 days after planting), however, the roots continued to accumulate nitrogen up to harvest time.

As was the case with dry-matter production, reductions in nitrogen fertilizer did not change the shapes of the nitrogen-uptake curves, however, the rates of uptake were lower.

Phosphorus Uptake

The effect of the treatments on uptake of phosphorus was similar to that which was observed in dry-matter production and nitrogen uptake. The total phosphorus uptake at the end of the season for all treatments in the study is shown in Figure 5. The high-yielding treatment resulted in maximum phosphorus uptake—100 pounds of P_2O_5 per acre. Phosphorus uptake was closely correlated with reductions in dry-matter production. For the low-yielding treatment, where no-nitrogen fertilizer was applied, the total uptake of phosphorus was only 62 pounds of P_2O_5 per acre.

Phosphorus in the plants for the well-fertilized treatments was about equally divided between the tops and roots or approximately 50 pounds of P_2O_5 per acre contained in each. As nitrogen fertilizer was reduced the phosphorus top-root ratio was also reduced.

Potash Uptake

Large amounts of potash (over 900 pounds of K_2O per acre) were taken up by sugar beets fertilized with nitrogen. The total potash uptake at the end of the growing season is given in Figure 6. No potash fertilizer was applied so that the entire uptake was derived from potash available in the soil. In previous work (3) no response has been obtained from potash application. Reductions in growth of the sugar beets resulted in less potash uptake. A low uptake of 440 pounds of K_2O per acre was obtained where no nitrogen was applied.

A high proportion of the total potash was contained in the tops and amounted to approximately 73 percent of the total for all of the well fertilized treatments. Thus, in the higher-yielding treatments, approximately 625 pounds of K_2O per acre were contained in the tops while about 240 pounds of K_2O per acre were contained in the roots.

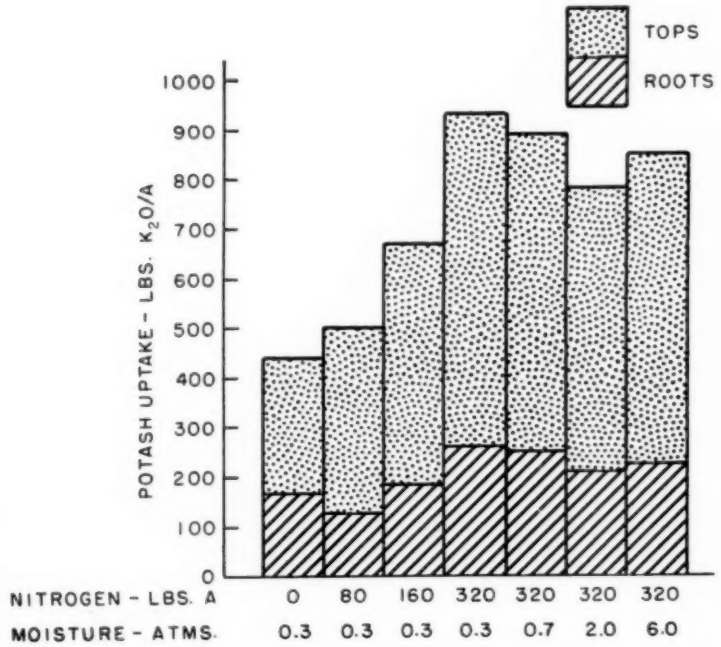

Figure 6.—The uptake of potassium by sugar beets under various nitrogen and soil-moisture treatments, Imperial Valley, California, 1954.

Summary

In a study made during the 1953-1954 sugar-beet season in Imperial Valley, California, the seasonal rate of growth and nutrient uptake by sugar beets was determined. Samples of beets were taken periodically throughout the growing season from an experiment having nitrogen and soil-moisture variables. When adequately fertilized with nitrogen and irrigated to yield 26 to 30 tons of beets per acre, beets produced 12 tons of dry matter per acre which contained over 400 pounds of nitrogen, 100 pounds of P_2O_5 and 900 pounds of K_2O. Increases in yield of beets, dry matter and uptake of nitrogen, phosphorus and potash were obtained with additions of nitrogen fertilizer even at the rate of 320 pounds of nitrogen per acre.

The major portion of the growth and nitrogen uptake by beets was made from January until the early part of May. After that time no further increase in top growth was obtained, however, the roots continued growth until harvest. As nitrogen application was increased, the top-root ratio increased.

This study shows that large amounts of dry matter are produced by a winter-grown sugar beet crop in the Imperial Valley. It emphasizes also the high amount of nitrogen used in growing a crop yielding 25 to 30 tons beets per acre and that the maximum rate of nitrogen uptake occurs from 90 to 215 days after planting for an October planted crop. In a nitrogen-fertility program for sugar beets it is therefore important that an adequate supply of nitrogen be made readily available to the crop just prior and during that period.

Reductions in the nitrogen fertility of a field up to 160 pounds of nitrogen per acre can be expected by removal of the beet roots for sugar processing. Similarly if the tops are removed an additional 200 to 250 pounds of nitrogen per acre would be lost. Phosphorus lost in the removal of the roots alone would be approximately 40 to 50 pounds of P_2O_5 per acre per acre.

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(2) BARTON, C. J. 1948. Anal. Chem. 20:1063.

(3) KRANTZ, B. A., and MACKENZIE, A. J. 1954. Response of sugar beets to nitrogen fertilizer in the Imperial Valley, California. Proc. Amer. Soc. Sugar Beet Tech. VIII (1): 36-41.

Identification of Genetically-Superior Individuals and the Prediction of Genetic Gains in Sugar Beet Breeding Programs[1]

LeRoy Powers[2]

The identification of genetically- superior individuals and the prediction of genetic gains should add materially to the effectiveness of the breeding programs with sugar beets and other crops. The purposes of this article are to present methods and formulas used to identify genetically-superior individuals and to predict genetic gains in segregating and heterogeneous populations. Applications of these methods and formulas are illustrated.

Literature Review

Wright (28)[3] discusses the genetic principles governing the rate of progress of livestock breeding. These principles are equally basic to plant breeding.

Dickerson and Hazel (3) and Lush (11) have developed methods and formulas for predicting genetic gains applicable to animal breeding. Robinson, Comstock, and Harvey (21) and Robinson, Mann, and Comstock (22) developed somewhat similar methods and formulas for predicting genetic gains in plant breeding.

The immediate genetic theory basic to the development of the methods of identifying genetically-superior individuals has to do with the partitioning of variance into its components and the partitioning method of genetic analysis. This is also true of the methods used in predicting genetic gains.

Fisher (4) was the first to provide a statistical method of partitioning variance into its environmental and genetic components. Fisher, Immer, and Tedin (6) elaborated on this method and gave several formulas for further partitioning genetic variance, using barley data to illustrate the application of the formulas. Wright (24, 25, 26, and 27) independently developed formulas and methods for studying variance components. These methods have been applied and extended by research workers in the animal and plant fields (see Lush, 11; Comstock and Robinson, 1; Robinson, Comstock, and Harvey, 19 and 20; Mather, 12; Lerner, 9; and Powers, 17).

Powers (14, 16, and 17) and Powers, Locke, and Garrett (18) have presented methods and formulas in addition to the above that are basic to the methods presented in this article for identifying genetically-superior individuals and predicting genetic gains in segregating and heterogeneous populations.

Lerner and Hazel (10) applied the method developed by Dickerson and Hazel (3) to egg production in poultry. The predicted gain per bird

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[1] Report of a study made by the Field Crops Research Branch, Agricultural Research Service, U. S. Department of Agriculture in cooperation with the Agronomy Section, Colorado Agricultural Experiment Station, and with the Beet Sugar Development Foundation. This paper has been approved for publication by the Station Director as Scientific Series Article No. 484.

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[3] Numbers in parentheses refer to literature cited.

per year was 5.28 eggs and the actual gain was 5.6 eggs per bird per year. It is obvious that the agreement between the rate of improvement expected from selection and that obtained is remarkably close.

Materials and Experimental Design

The characters studied were percentage sucrose and weight per root in sugar beets (*Beta vulgaris* L.). The experiment was conducted during the growing period of 1955.

Six populations were included in the study. The F_1 hybrid resulted from crossing two inbred lines, 50-406 with 52-307. GW359-52R is the variety being grown by farmers producing beets for the Great Western Sugar Company in the Rocky Mountain Region. SP 53104-0 is a United States Department of Agriculture production under development as a variety carrying resistance to curly top and leaf spot. US 201 is not a commercial variety but a closebred strain extremely high in leaf-spot resistance and is very valuable as a parent to furnish genes conditioning resistance to the organism causing this disease. The inbred lines 50-449 and 52-305 were produced by the late G. W. Deming. The exact number of generations of inbreeding is not known. However, Mr. Deming stated that 52-305 had resulted from many generations of inbreeding and was included in the studies because he believed it to be relatively homozygous.

Inbred line 52-305 and the F_1 hybrid were included in the experiment to obtain a measure of environmental variability and to furnish some measure of the reliability of the methods used. The inbred line 50-406 has recessive-green hypocotyls and 52-307 has dominant-red hypocotyls. Hence, the F_1 plants produced by seed harvested from 50-406 could be identified by hypocotyl color. During thinning, the green hypocotyl plants were eliminated, thus insuring that only F_1 hybrid plants were left. At the time of harvest, all roots were found to have pink buds, and in this manner the F_1 cultures were checked for possible self- or intra-fertilized plants.

In planting the different cultures of the experiment, the rows were spaced 40 inches apart and subsequently the beets were thinned to a spacing of 20 inches between beets within the row. The wide spacing between and within rows was used to minimize competition between beets. At time of thinning each culture was reduced to 12 plants and only 8 plants were harvested per culture. Any beets showing visual evidence of disease, either before or after being pulled, were discarded.

The field design of the experiment was a randomized complete block composed of two parts. The first part included the six populations listed in Table 1, and the second part included 52-307 in place of the F_1 hybrid. The first part was composed of 20 blocks and the second part of 60 blocks. Since, for the second part of the experiment, eight roots were not available for all plots the number was reduced, by random elimination, to six before analysing the data. Hence, there was a total of 160 plants per population available for statistical analysis in the first part of the experiment and 360 available for analysis in the second part of the experiment. Five of the populations occur in both parts, making a total of 520 plants for these populations. The present article reports on the analysis of the populations involving 160 individuals and those involving 520 individuals.

Table 1.—Population Frequency Distributions Expressed in Percentages for Percentage Sucrose[1] [2]

Population	Upper limit of Class in Percent														
	0 to 6.75	7.50	8.25	9.00	9.75	10.50	11.25	12.00	12.75	13.50	14.25	15.00	15.75	16.50	17.25 & >
	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%
F_1 hybrid						1.9	7.5	21.9	14.4	28.8	15.6	8.7	1.2		
GW359-52R		0.6	0.6	1.9	0.6	8.8	10.0	15.6	21.9	14.4	13.1	5.0	3.8	2.5	1.2
SP 53104-0	1.2	2.5	5.0	1.9	8.7	8.1	14.4	21.9	11.2	14.4	6.9	3.8			
US 201	1.9	2.5	6.2	5.6	8.8	15.6	20.6	19.4	5.0	8.1	3.8	0.6	1.9		
50-449	0.6	2.5	5.0	7.5	8.1	21.3	20.6	18.1	7.5	5.6	1.3	1.9			
52-305					0.6	6.9	12.5	18.8	25.6	23.1	11.9		0.6		

[1] Number of plants per population = 160.

[2] The percentage of any given frequency distribution enclosed in brakets is the proportion identified as genetically superior.

Identification of Genetically-Superior Individuals for Percentage Sucrose

The frequency distributions of the populations for percentage sucrose having N values of 160 are listed in Table 1. In reality identification of genetically-superior individuals and the prediction of genetic gains involve analyses of these frequency distributions. The frequency distributions are expressed in percent rather than number of plants, and the upper limits of the classes are given. By so doing the analyses of the data are facilitated.

One of the first steps in analysing the frequency distributions is to partition the variances into their components. To do this it is necessary to have a measure of the environmental variability. Inbred line 52-305 and the F_1 hybrid 50-406 x 52-307 were included in the experiment to measure the environmental variability. An examination of Table 1 reveals that the ranges (number and values of classes in which individuals occur) are not greatly different for the frequency distributions of 52-305 and the F_1 hybrid and are considerably less than those for the other populations. It appears that either, or both, of these populations furnish a satisfactory estimate of the environmental variability. Whether this assumption is correct will be determined as the analyses of the data proceed. For percentage surcrose, data from inbred 52-305 are used to estimate the environmental variance and those from the F_1 hybrid to give a practical check on the methods and formulas employed.

It is important to determine which populations have genetic variances. Those not showing any genetic variability would not be expected to possess any genetically-superior individuals nor would genetic gains be possible by breeding within them. Hence, the data from 52-305 and the F_1 hybrid compared with the data from the segregating populations serve somewhat as a practical check on the reliability of the methods employed to identify genetically-superior individuals and to predict genetic gains.

The means, total variances, and genetic variances of populations for percentage sucrose are listed in Table 2. The information in this table indicates whether there are differences between means of populations, in which populations genetically-superior individuals occur, and finally in which populations it is possible to make genetic gains by application of suitable breeding methods.

As given by footnote 2 of Table 2 the F value obtained by analysis of variance is 21.13 for differences between means of populations. That required for significance at the one percent level is 4.40. There are differences between means of populations as regards percentage sucrose. At least, the means fall into two classes; the F_1 hybrid, GW359-52R, and 52-305 comprising one class, and SP 53104-0, US 201, and 50-449 comprising the other class.

The total variance of 52-305 listed in Table 2 is used as an estimate of the environmental variance. The genetic variances listed in the fourth column of this table are obtained by subtracting this estimate (1.1533) from the total variances of column 3.

A comparison of the genetic variances with their standard errors reveals that all except the variance of the F_1 hybrid are significantly different from zero. The estimated genetic variance of the F_1 hybrid should not be significantly different from zero if both parents are relatively homozygous. This confirms that either 52-305 data or the data of the F_1 hybrid, or both, are suitable for estimating the environmental variability of percentage sucrose.

Table 2.—Means, Total Variances, and Genetic Variances for Percentage Sucrose and Populations[1].

Population	Mean[2] Sucrose	Variance[3]	
		Total	Genetic
	Percent		
F_1 hybrid	12.8	1.2578±0.1411	0.1045±0.1914
GW359-52R	12.5	2.9929±0.3357	1.8396±0.3597
SP 53104-0	11.3	3.7125±0.4164	2.5592±0.4360
US 201	10.7	3.7695±0.4228	2.6162±0.4421
50-449	10.7	2.6376±0.2958	1.4843±0.3229
52-305	12.3	1.1533±0.1293	——— ———

[1] The number of plants per population = 160.

[2] The F value for populations is 21.13, the degrees of freedom are 5, and for populations times replications, which variance was used as an estimate of error, the degrees of freedom are 95. The mean square used in calculation of the F value for populations is derived from culture totals (total of 8 plants).

[3] These are the within population variances calculated from individual plant data. The standard errors of the total variances are calculated from a formula given by Fisher (5, page 78). The standard errors for the genetic variances are calculated from the well known formula for the standard error of a difference.

In identifying genetically-superior individuals it is assumed that the proportion of individuals in each class of Table 1 can be estimated and the deviations of the estimates from the obtained frequency distributions will not be greater than expected by chance. This assumption can be tested for the F_1 hybrid and 52-305 populations by testing for agreement using the chi square frequency distribution. The means of the population under consideration, the standard error calculated from the environmental variance (variance of 52-305, see Table 2), and Pearson's (13) normal probability tables are employed. For details of the method see Powers (15) and Leonard, Mann, and Powers (8). For both of these populations the deviations from the calculated and obtained frequency distributions are no greater than expected due to chance. All of the data necessary to carry out the calculations are given in Tables 1 and 2.

The finding that the frequency distributions based on environmental variation can be predicted within the limits of random sampling, justifies proceeding with the analysis pertaining to the identification of individuals genetically superior and to prediction of genetic gains. To do this it is necessary to determine an upper class beyond which the odds are great that individuals will not occur due solely to environmental variability. That is, the odds are great that the individuals occurring beyond the upper limit of

this class are genetically superior to the average of the population. Stated in still another way these individuals have values greater than those delimited by chance fluctuations due to the environmental variability. The methods and formulas used in determining the upper limit of the class beyond which only individuals genetically superior to the average of the population would be expected to occur are given in a previous publication (Powers, 16). Certain minor modifications and extensions of the use of these methods and formulas are necessary in identifying genetically-superior individuals and in predicting genetic gains. Therefore their application to percentage sucrose will be given in some detail.

The formula for predicting the mean of sub-group frequency distributions is $y = z - s_d x'$ (Powers, 16). In identifying genetically-superior individuals the z values given in Table 3 are obtained by application of the formula $z = \bar{x} + s_d x'$. This formula is easily derived from the formula $y = z - s_d x'$, in which $\bar{x}$ is substituted for y. In the application of these formulas the symbols used and those given in Table 3 have the following connotations.

y = predicted mean
$\bar{x}$ = obtained mean
z = upper limit of class
s_d = estimated standard error calculated from the environment variance of 52-305 (total variance of Table 2).
$p = 1 - q$
$q = \frac{1}{2}(1 + a)$ Pearson's (13) tables
$x' = x$ of Pearson's (13) tables

Table 3.—Percentage of Identified-Genetically-Superior Individuals and Estimated Means of Sub-Group Frequency Distributions in Which These Individuals Occur For Percentage Sucrose and Populations[1, 2].

Populations	Value of z for p = 0.003	Value z Used	Superior Individuals[3]	Value of x'	Mean			
					Obtained $\bar{x}$	Predicted, y		
						Superior Individuals[3]	12.5%	37.5%
	Percent	Percent	Percent		Percent	Percent	Percent	Percent
F_1 hybrid	15.70	15.75	0.0	——	12.8	——	——	——
GW359-52R	15.40	15.75	3.7	1.79	12.5	13.8	14.5	15.4
SP 53104-0	14.20	14.25	3.8	1.78	11.3	12.3	13.0	13.9
US 201	13.60	14.25	2.5	1.96	10.7	12.1	13.0	13.9
50-449	13.60	14.25	1.9	2.07	10.7	12.0	13.0	13.9
52-305	15.20	15.75	0.0	——	12.3	——	——	——

[1] The number of plants per population = 160.

[2] The variance of 52-305 is 1.1533 (see Table 2) and the standard error (s_d) is 1.0739. This variance and this standard error were used as estimates of the environmental variance and environmental standard error, respectively.

[3] These means are estimated from the percents listed under the column heading "Superior individuals" and the superior individuals are the percentages of individuals in classes of Table 1 falling beyond the values of z used (3rd column of Table 3).

In identifying genetically-superior individuals it is necessary to determine the value of p to be employed. The value of p that should be used is dependent upon the number of individuals in the population and the intensity of the selection to be practiced. Another way of stating the problem that may help to clarify this point is as follows: The decision as to what value of p to use is based on the number of individuals the investigator wishes to select for further study and breeding, and the number of genetically-superior individuals it is possible to identify in the population under consideration.

For example, if a p value of 0.003 is employed, as shown previously the value of z may be calculated from the formula $z = x + s_d x'$. The calculations for GW359-52R are as follows:

$x = 12.5$ (see Table 3)

$s_d = 1.0739$ (see footnote of Table 2)

$p = 0.003$ (chosen value)

$q = 0.997$ (1-p)

$x' = 2.70$ (value of x in Pearson's (13) tables for $\frac{1 + a}{2}$ equals 0.997)

$z = 12.5 + (1.0739 \times 2.70) = 15.40$ as given in the second column of Table 3

The values of z for the other population given in the second column of Table 3 are calculated in a similar manner.

Since, in this example, p was chosen as 0.003 only one individual in a population of 333 would be expected to equal or exceed the value of 15.40 percent sucrose. Hence, the probability of two individuals in a population of 160 exceeding 15.40 percent sucrose is very small. Consequently the p value of 0.003 should be satisfactory for studying the frequency distributions of Table 1 providing, due to genetic superiority, there are a sufficient number of individuals exceeding the value of z used.

The value of z as calculated above seldom corresponds to an upper class limit, as is necessary to identify the genetically-superior individuals in frequency distributions such as those listed in Table 1. From an examination of the frequency distribution of GW359-52R given in Table 1 it can be seen that the calculated z value (15.40) falls into the class having an upper limit of 15.75 percent sucrose. Hence, the value of z selected is 15.75 as shown in the third column of Table 3. The other values of z listed in this column were determined in a similar manner. It should be pointed out that if the investigator desires to work with calculated values of z, such as 15.40, he can do so by referring to the data from which the frequency distributions were compiled.

The value of 3.7 listed in the fourth column of Table 3 is the percentage of individuals in the frequency distribution of GW359-52R that exceeded 15.75 percent sucrose. As the fourth column heading indicates, the percentages listed under it are those cumulated for all the classes whose upper limits exceed the value of z used.

For GW359-52R, the number of individuals having an upper class limit greater than 15.75 is 6 (3.7 percent of 160). The chances are very good that all six of these individuals are genetically superior as regards percentage sucrose, and it is reasonably certain that at least five of them fall into this category. By labeling all beets at time of harvest with culture and plant number these individuals can be selected for further use in the breeding program and for further studies.

It is apparent that the percentages listed in column 4 of Table 3 are the proportions of identifiable-genetically-superior individuals in the respective populations. As stated previously, if the beets are labeled at time of harvest the procedures and calculations outlined provide a means of identifying some of the genetically-superior individuals. It is equally apparent that these are not all the genetically-superior individuals in the population. In all probability they are not even a preponderant proportion of such individuals.

Predicting Genetic Gains in Percentage Sucrose

The percentages of identifiable-genetically-superior individuals listed in the fourth column of Table 3 provide a basis of predicting genetic gains in percentage sucrose. It is clear that these individuals are members of a sub-group frequency distribution having a mean larger than that of the population in which they occur. Environmental variability and probably to some extent genetic variability are causing them to fluctuate and hence form a sub-group within the population frequency distribution. The problem has resolved into one of estimating the means of these sub-group frequency distributions of which these genetically-superior individuals are members.

The formula employed to predict the means of these sub-group frequency distributions is $y = z - s_d x'$ (see Powers, 16) and the connotations of the symbols are as given previously in this article. The number of individuals in this sub-group frequency distribution is not known, and therefore the proportion that the genetically-superior individuals are of this sub-group is not known. However, what proportion they are of the respective populations in which they occur is known. For GW 359-52R the identifiable-genetically-superior individuals constitute 3.7 percent of the population.

Using the percentage for GW359-52R a conservative estimate (underestimate) of the sub-group mean can be calculated. As stated above, the formula employed is $y = z - s_d x'$. The calculations are as follows: Converted to decimal fractions the proportion of genetically-superior individuals is 0.037. This is the value of p. The value of q is 1-p, or 0.963, and it is designated as $\frac{1}{2}$ (1 + a) in Pearson's (13) tables. As taken from Pearson's tables the corresponding value of x′ is 1.79. The x′ values for all populations are listed in the fifth column of Table 3. The value of z used for GW359-52R is 15.75 (see Table 3). This is a conservative estimate of z, because, as can be determined from Table 1, 1.2 percent of the population exceeded 16.50 percent sucrose. By substituting the proper values in the formula given at the beginning of this paragraph, $y = 15.75 - (1.79 \times 1.0739)$. By completing the calculations a value of 13.8 percent sucrose is obtained, as listed in the seventh column of Table 3. The predicted genetic gain in

percentage sucrose for GW359-52R is 13.8 minus 12.5 (column 7 minus column 6), or 1.3 percent. The values for the other populations are calculated similarly.

The values listed under columns 8 and 9 of Table 3 (heading 12.5% and 37.5%, respectively) were obtained by substituting 12.5% and 37.5% for the values of column 4 of Table 3 and carrying out the calculations as given in the immediately preceding paragraph. The values listed in column 9 compared with the obtained means (column 6) represent maximum genetic gains that may be possible by breeding within the populations listed in Table 3. The genetic levels that can be reached for these populations, by appropriate methods of breeding, probably lie somewhere between the values listed in column 7 and those listed in column 9.

The values for percentage sucrose listed in column 7 of Table 3 compared with those given in column 6 show the expected genetic gains based on a conservative estimate of the means of the sub-group frequency distributions. These gains for the populations starting with GW359-52R and going down the table in order are 1.3, 1.0, 1.4, and 1.3 percents, respectively. The increases expressed as percentages of the obtained means are 10.4, 8.8, 13.1, and 12.1 percents, respectively.

Identification of Genetically-Superior Individuals and Prediction of Genetic Gains for Weight per Root

The methods and formulas used in the identification of genetically-superior individuals and to predict genetic gains for weight per root are essentially the same as those for percentage sucrose. The differences that do exist are attributable to the positive relation between the means and environmental variances for weight per root. Hence the variances of the non-segregating populations cannot be used directly as an estimate of the environmental variance for the segregating populations. For such characters as weight per root, regression may be used to estimate the environmental variances of the segregating populations (see Powers, 14). This method of estimation assumes a straight line relation between the means and environmental variances. Consequently, to employ this method two points on the line are essential. In the present study these are furnished by the means and variances of the F_1 hybrid and inbred line 52-305. When studying characters that exhibit heterosis, such as weight per root, it is desirable to include at least one F_1 hybrid and at least one inbred line. This usually assures that two rather widely separated points on the regression line will be available for estimating the environmental variances of the segregating populations.

The population frequency distributions for weight per root are given in Table 4. The smallest range is for 52-305 and the greatest range is for GW359-52R. The range for the F_1 hybrid is considerably greater than that for 52-305 and the frequency distribution lies in classes having a greater weight per root. This is to be expected if both the mean weight per root and the environmental variance are greater for the F_1 hybrid. As was the case for percentage sucrose, the identification of genetically-superior individuals and prediction of genetic gains for weight per root involve analyses of the frequency distributions which are listed in Table 4.

Table 4.—Population Frequency Distributions Expressed in Percentages for Weight Per Root[1,2].

Population	Upper Limit of Class in Pounds																		
	0 to 0.75	1.50	2.25	3.00	3.75	4.50	5.25	6.00	6.75	7.50	8.25	9.00	9.75	10.50	11.25	12.00	12.75 & >
	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%
F1 hybrid				7.5	13.1	25.0	21.9	16.3	10.0	4.4	1.2	0.6					
GW359-52R		1.9	0.6	1.9	3.7	11.9	12.5	13.1	12.5	10.0	10.6	8.8	4.4	5.0	0.6	0.6	1.9
SP 53104-0			4.4	10.6	13.1	24.4	16.9	16.3	6.9	6.2		1.2					
US 201	3.1	11.3	15.0	26.9	18.8	14.4	8.1	0.6	1.2	0.6							
50-449		5.6	11.9	33.8	23.8	16.2	6.2	1.9			0.6						
52-305		3.1	45.7	45.0	5.6	0.6											

[1] Number of plants per population = 160.

[2] The percentage of any given frequency distribution enclosed in brackets is the proportion identified as genetically superior.

Again, as for percentage sucrose, the analyses of these frequency distributions for weight per root assume that the proportion of individuals in the different classes of the frequency distribution can be estimated by employing Pearson's (13) normal probability tables and formulas given by Powers (16). These formulas for calculating the theoretical frequency distributions make use of the obtained means and environmental standard errors. Whether the calculated theoretical proportion of individuals in the different classes agrees with those obtained can be determined for the F_1 hybrid and 52-305 populations. For both populations applications of chi square for agreement revealed that the deviations between the obtained and calculated proportions in the different classes of the frequency distributions are no greater than expected by chance. All the data necessary for making the test are given in Tables 4 and 5. This finding justifies proceeding with the analyses of the data.

The means, total variances, within-culture variances, within-culture environmental variances, and within-culture genetic variances for weight per root are listed in Table 5.

Table 5.—Means, Total Variances, Within-Culture Total Variances, Within-Culture Environmental Variances, and Within-Culture Genetic Variances for Weight Per Root and Populations[1].

Population	Mean	Variance			
		Total[2]	Within Cultures[3]		
			Total	Environmental	Genetic
	Lbs.				
F_1 hybrid	4.74	1.4194±0.1592	1.0145±0.1327	1.0145±0.1327	——— ———
GW359-52R	6.44	5.1248±0.5748	5.0596±0.6429	1.5729±0.2182	3.4867±0.6723
SP 53104-0	4.56	1.9520±0.2189	1.7879±0.1871	0.9469±0.1296	0.8410±0.1634
US 201	2.94	1.6270±0.1825	1.4861±0.1992	0.4196±0.0819	1.0665±0.1841
50-449	3.09	1.1246±0.1261	1.0139±0.1789	0.4417±0.0700	0.5721±0.1688
52-305	2.29	0.2349±0.0263	0.1825±0.0374	0.1825±0.0374	——— ———

[1] The number of plants per population = 160.

[2] The standard errors of these variances are calculated from a formula given by Fisher (5, page 78).

[3] The standard errors of these variances are calculated from the data, variances for cultures within populations. For the details of calculating the variances of these variances see Powers (17, pages 9 and 10).

The t test in which standard errors were calculated for each population from the data within each population shows that there are significant differences between means of populations. However, since there is a positive relation between the means and variances, the results from the analysis of variance must be interpreted with this in mind. The analysis of variance together with the results from the t test seem to justify the following conclusions. The F values obtained from an analysis of variance led to the same conclusions. The means compose four levels of weight per root; GW359-52R composes one level, the F_1 hybrid and SP 53104-0 another, US 201 and 50-449 another, and 52-305 the fourth level.

There are four different columns of variances listed in Table 5. These are given to illustrate the difference in estimating the genetic variances for percentage sucrose in which there is no relation between the means and environmental variances and weight per root in which there is a positive relation between the means and variances.

It will be remembered that in Table 2 the genetic variances were calculated by subtracting the total variance of 52-305 from the total variances of the other populations. The variances given in the 3rd column of Table 5 correspond to the variances given in the third column of Table 2. The within-culture total variances given in the fourth column of Table 5 were calculated from the eight plants of each culture of a given population. The errors were calculated by standard methods using the 20 estimated variances for a given population. It is interesting to note the rather close agreement between the errors for the total variances (column 3) calculated by use of Fisher's formula (5) and those (column 4) obtained from the data for the within-culture total variances.

Likewise the within-culture environmental variances (column 5) were calculated on the basis of cultures, and the standard errors were obtained by use of standard methods applied to the 20 variances of a given population. Finally, on the basis of cultures, the within-culture genetic variances were calculated by subtracting the corresponding within-culture environmental variance from the within-culture total variance. Again the standard error for any given population was calculated from the 20 genetic variances so estimated.

Table 6.—Percentage of Identified-Genetically-Superior Individuals and Estimated Means of Sub-Group Frequency Distributions in Which These Individuals Occur for Weight Per Root and Population[1].

Population	Value of z Used[2]	Environmental Standard Error[3]	Superior Individuals	Value x'	Mean			
					Obtained $\bar{x}$	Predicted, y		
						Superior Individuals	12.5%	37.5%
	Lbs.		%		Lbs.	Lbs.	Lbs.	Lbs.
F_1 hybrid	9.00	1.1914	0.0	——	4.74	——	——	——
GW359-52R	11.25	1.4971	2.5	1.96	6.44	8.32	9.53	10.77
SP 53104-0	8.25	1.1543	1.2	2.25	4.56	5.65	6.92	7.88
US 201	5.25	0.7410	2.4	1.97	2.94	3.79	4.40	5.01
50-449	6.00	0.7884	0.6	2.49	3.09	4.04	5.09	5.75
52-305	4.50	0.4847	0.0	——	2.29	——	——	——

[1] The number of plants per population = 160.

[2] The value of p for the values of z used are equal to or less than 0.001.

[3] The variances giving rise to these standard errors for the heterogeneous populations are calculated from the mean of the respective population and the means and total variances (column 3 of Table 5) of the F_1 hybrid and 52-305 (for method see Powers, 14).

By comparing the genetic variances with their standard errors it is clear that they are significantly different from zero for GW359-52R, SP 53104-0, US 201, and 50-449. In these populations it should be possible to identify genetically-superior individuals and to predict genetic gains.

The percentage of identified-genetically-superior individuals and estimated means of the sub-group frequency distributions in which these individuals occur are given in Table 6. The variances giving rise to the standard errors listed in the third column of Table 6 are calculated by regression involving the mean of the respective population and the m and b constants in the formula $y' = mx+b$. The symbols in the formula have the following connotations:

y' = environmental variance
m = the slope of the regression line
b = the point of intercept of the regression line and the ordinate
x = the mean of the population whose variance is being estimated

In application of the above formula m and b are calculated from the means and variances of the F_1 hybrid and 52-305. For details of the method for estimating the environmental variances of the heterogeneous populations see Powers (14).

The genetically-superior individuals identified are those composing the percentage values listed in the fourth column of Table 6. The number of identified-genetically-superior individuals for GW359-52R and US 201 populations is four (0.025 X 160), for SP 53104-0 is two, and for 50-449 is one. Since the value of p used in calculating the percentage of identifiable-genetically-superior individuals was 0.001 the chances are good that most, if not all, of the 11 individuals representing the percentages listed in column 4 of Table 6 are genetically superior. The chances are very small that two of them would occur in these classes due to environmental variability.

A conservative estimate of the genetic gains possible by application of the proper breeding methods is obtained by comparing the values listed under mean and obtained, with those listed under mean and superior individuals. The increases expected for the heterogeneous populations starting with GW359-52R and proceeding down Table 6 are 29.2, 23.9, 28.9, and 30.7 percent, respectively. Hence, according to the conservative estimates, decided advances can be made in weight per root. The increased levels of yield are even higher for the columns listed under 12.5 percent and 37.5 percent. However, these latter levels of yield would be much more difficult to attain.

Percentage Sucrose and Weight per Root Considered Simultaneously

So far the analysis of the data has considered the identification of genetically-superior individuals for either percentage sucrose or weight per root, but not both simultaneously. In any breeding program both characters must be taken into consideration. At least, the breeder attempts to maintain the level of one of these characters while improving the other.

Table 7.—Frequency Distributions of GW359-52R Expressed in Percentages for Percentage Sucrose and Weight per root,[1],[2],[3]

Weight Per Root Upper Limit of Class in Pounds	Percentage Sucrose Upper Limit of Class in Percent																	Total
	0 to 7.50	8.25	9.00	9.75	10.50	11.25	12.00	12.75	13.50	14.25	15.00	15.75	16.50	17.25 & >				
	%	%	%	%	%	%	%	%	%	%	%	%	%	%				%
0 to 1.50								0.2	0.2	0.2	0.2			0.2				1.0
2.25								0.2	0.6	0.2		0.4						1.4
3.00						0.2		0.4	0.7	0.4	0.2		0.2	0.2				2.3
3.75					0.2	0.2	0.4	0.8	1.1	1.5	1.3	1.2	0.2					6.9
4.50				0.2	0.4	0.6	1.0	1.7	2.3	1.5	2.1	1.3		0.2				11.3
5.25	0.2	0.4	0.4		0.4	0.8	1.2	2.3	3.3	1.9	1.3	0.8	0.5					13.5
6.00					0.6	0.8	1.9	2.5	2.5	3.1	1.1	1.1	0.4					14.0
6.75			0.2	0.2	0.4	0.2	2.7	2.5	2.7	1.9	1.1	0.8	0.2					12.9
7.50			0.2	0.2	0.6	1.3	2.1	1.9	1.2	2.5	1.0							10.8
8.25			0.2	0.3	0.7	0.6	1.3	2.9	1.2	1.2			0.2					8.6
9.00				0.2	0.8	1.7	1.1	1.2	1.1	0.6								6.7
9.75		0.2		0.2		1.1	0.8	0.9	0.8	0.4								4.4
10.50					0.6	0.6	0.7	0.6	0.2	0.2								2.9
11.25		0.2			0.4		0.2	0.2										1.0
12.00					0.3	0.2	0.2	0.4			0.2							1.3
12.75 & >	0.2				0.2	0.4			0.2									1.0
Total	0.4	0.8	1.0	1.3	5.6	8.7	13.6	18.7	18.1	15.4	8.5	5.6	1.7	0.6				100.0

[1] The number of plants = 520.
[2] The mean percent sucrose = 12.7.
[3] The mean weight per root = 6.19 lbs.

The study of percentage sucrose and weight per root simultaneously involves the analysis of the frequency distribution for both characters. The frequency distribution for percentage sucrose and weight per root is given in Table 7. From Table 3 it can be determined that for GW359-52R the identified-genetically-superior individuals exceeded 15.75 percent sucrose and from Table 6 that the identified-genetically-superior individuals exceeded 11.25 pounds per root. The percent of this population meeting this requirement for percentage sucrose is 3.7, and for weight per root is 2.5. The expected proportion of the population exceeding the upper class limits for both characters would be the product of their separate probabilities on the basis that they are independent. In this case it is 0.037 times 0.025 or 0.000925. If these two characters are independent, approximately one individual in a thousand would be expected to fall into categories that would identify them as genetically superior for both characters. Hence, in a population of 520 individuals, none would be expected and none were obtained, as can be seen from Table 7.

An examination of the frequency distribution for GW359-52R given in Table 7 shows that there is a negative relation between percentage sucrose and weight per root. How this negative relation affects the probability of obtaining some genetic gain in both characters can be determined to some extent by studying that portion of the frequency distribution delimited by a solid black line. The solid black line delimits those individuals that exceed the limits of the class in which the averages of the characters fall. The percent of the individuals for percentage sucrose falling in this area of the frequency distribution is 36.7 and for weight per root is 49.9. The expected percent for both characters on the basis of independence is 18.3. The percent obtained is 10.8. Among 520 individuals and on the basis of independence, 95 would be expected to fall within the area of Table 7 delimited by the solid line. The number obtained is 56. The chi square value for testing agreement between the two ratios (425:95 and 464:56) has a p value less than 0.01. The entire frequency distribution confirms that the two characters are not independent. The relation is negative.

It has been shown that on the basis of independence, only one beet in a thousand could be identified as genetically-superior for both percentage sucrose and weight per root. This means that on the basis of independence, to obtain 10 such beets would require a population of 10,000. Since the relation is negative between percentage sucrose and weight per root more individuals would be required. An examination of the data of Table 7 indicates that the negative relation between percentage sucrose and weight per root in this population is reasonably linear. It is apparent that very large populations would be required to identify fifty or more individuals in GW359-52R genetically superior for both characters.

Whether this same negative relation between percentage sucrose and weight per root exists for other populations and, if so, whether the regression is linear within and between populations should be determined. To obtain some information the total covariances and genetic covariances were calculated for all the segregating populations. They are listed in Table 8.

Table 8.—Within Cultures Total Covariances and Genetic Covariances for Percentage Sucrose and Weight Per Root[1].

Population	Covariance within cultures	
	Total	Genetic
F_1 hybrid	—0.2133±0.0555	——— ———
GW359-52R	—1.8109±0.3615	—1.6520±0.3657
SP 53104-0	—0.3787±0.1883	—0.2197±0.1908
US 201	—0.2059±0.1299	—0.0470±0.1393
50-449	—0.3626±0.1356	—0.2036±0.1473
52-305	—0.1046±0.0235	——— ———

[1] The number of plants per population = 160.

The covariances, both total and genetic, were calculated from individual plant data within cultures and populations. Their standard errors were calculated from the covariances within a population (for details of the method see Powers 17, pages 9 and 10). The total covariances for the F_1 hybrid and 52-305 populations are environmental covariances. They are significantly different from zero, as the covariance for the F_1 hybrid is 3.84 times its standard error and that for 52-305 is 4.45 times its standard error. In fact, with the possible exception of US 201, all of the total covariances are significantly different from zero.

From the data given in Table 8 and as determined by a comparison with their standard errors the only genetic covariance significantly different from zero is the one for GW359-52R. However, the genetic covariances for the other three populations are negative and hence furnish some evidence that even in these populations a slight negative relation may exist between percentage sucrose and weight per root. It is clear that the degree of the relation is similar for SP 53104-0, US 201, and 50-449; but of very little importance as compared with the extent to which this relation exists in the GW359-52R population. An understanding of the biological phenomena responsible for this difference between GW359-52R and the other segregating populations may be of fundamental importance to the sugar beet breeding program.

The frequency distributions for other populations involving percentage sucrose and weight per root may furnish some information pertaining to this problem. Such a frequency distribution for US 201 is given in Table 9. Again the solid line delimits individuals falling into classes beyond those in which the means occur. The second solid black line within this larger group delimits the classes in which it would be possible to identify the individuals genetically-superior for both characters, if any occur. As was also true for GW359-52R, it was not possible in the US 201 population to identify any individuals genetically superior for both characters. On the basis of independence of the two characters, only six such individuals would be expected in a population of 10,000. Hence, none would be expected in a population of 520 individuals and as can be seen none occurred.

Table 9.—Frequency Distributions of US 201 Expressed in Percentages for Percentage Sucrose and Weight Per Root[1, 2, 3].

Weight Per Root Upper Limit of Class in Pounds	Percentage Sucrose Upper Limit of Class in Percent														
	0 to 6.75	7.50	8.25	9.00	9.75	10.50	11.25	12.00	12.75	13.50	14.25	15.00	15.75	16.50 & >	Total
	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%
0 to 0.75						0.2	0.3	0.2		0.2	0.2			0.2	1.3
1.50	0.2			1.0	0.8	1.1	1.0	1.7	0.4	0.9	0.6	0.4	0.7		8.8
2.25	1.0	0.6	1.0	1.0	1.3	1.7	2.1	3.8	1.5	1.0	1.2				16.2
3.00	0.6	0.6	1.2	1.5	1.9	3.7	4.8	6.5	2.7	1.7	1.5	0.4	0.4		27.5
3.75		0.2	0.4	1.5	2.3	2.3	3.3	4.2	2.1	2.5	1.4	0.6			20.8
4.50			0.4	0.6	1.5	2.9	2.5	2.5	1.3	1.1	0.4	0.6			13.8
5.25				0.4	0.4	1.0	1.1	1.5	0.6	1.7		0.2			6.9
6.00					0.2	0.2	0.8	0.8	0.4		0.7				3.1
6.75						0.2	0.2	0.4				0.2			1.0
7.50						0.4									0.4
8.25 & >										0.2					0.2
Total	1.8	1.4	3.0	6.0	8.4	13.7	16.1	21.6	9.0	9.3	6.0	2.4	1.1	0.2	100.0

[1] The number of plants = 520.
[2] The mean percent sucrose = 11.1.
[3] The mean weight per root = 3.02 lbs.

Another interesting fact concerning the frequency distribution given in Table 9 is that for weight per root 2.7 percent of the population fall into those classes identifying them as genetically superior as regards weight per root and superior to the mean as regards percentage sucrose. For percentage sucrose the situation is quite different as all seven (1.3 percent of 520) of the individuals identified as genetically superior for percentage sucrose fall into classes having values lower than the mean for weight per root. This indicates that within that portion of the population having higher percentage sucrose the same negative relation noted between this character and weight of root for GW359-52R might exist for US 201. Since GW359-52R has both higher percentage sucrose and greater weight per root than US 201, this raises the question whether a threshold exists beyond which the negative relation between percentage sucrose and weight per root becomes pronounced.

If such were the case and most of the individuals for the three populations other than GW359-52R were to fall below this threshold, the small negative and statistically non-significant genetic covariances noted might be expected. Likewise, this same tendency of the identifiable-genetically-superior individuals for percentage sucrose to have lower weight per root is evident in the frequency distributions of populations SP 53104-0 and 50-449.

Another biological phenomenon that needs to be considered as a possible factor in bringing about the relation noted between percentage sucrose and weight per root is that the physiology of root growth and formation of sucrose is such that some of the physiological-genetic processes favorable to increased weight per root are not favorable to increased percentage sucrose. The fact that the environmental covariances for the F_1 hybrid and 52-305 populations are significantly different from zero (Table 8) would tend to support this explanation. If thresholds are involved, such a fact would lend further support to the conclusion that the physiology of the plant at certain levels of weight of root or certain levels of percentage sucrose does not favor both increased weight per root and increased percentage sucrose.

The frequency distribution of GW359-52R given in Table 7 does not furnish evidence of a threshold operating within this population, as the regression involving percentage sucrose and weight per root seems to be linear. In other words, the relation noted seems to exist to about the same degree throughout the population. It should be noted that the lower levels of percentage sucrose and weight per root are well within those of the other three segregating populations. This finding does not support the theory of a threshold at which increase in one character is associated with a decrease in the other character.

Discussion

Negative Genetic Covariances

Of interest are the possible explanations for the negative genetic covariance between percentage sucrose and weight of root in GW359-52R. For other segregating populations a tendency for this same relation was

noted only among those plants having high percentage sucrose. The data presented in this study do not provide a full, nor perhaps adequate, hypothesis to explain this relation. Even though evidence furnished by the data are not conclusive they should be of value in helping to clarify the problems involved and in designing further experiments.

The possibility of thresholds, at which the negative relation noted for percentage sucrose and weight per root becomes pronounced, has already been mentioned. The fact that percentage sucrose and weight per root are higher for GW359-52R and the negative relation is much more pronounced is evidence that thresholds may be playing some part. The frequency distributions of GW359-52R and other populations show considerable overlapping. Then, that thresholds are not the sole casual phenomenon is indicated by the apparent linearity of regression of these two characters (see Table 7) throughout the frequency distribution of the GW359-52R population. For other segregating populations, the tendency for this same negative relation to exist among the plants having the higher percentages of sucrose is further evidence that thresholds may be playing some part.

Another possible explanation for the negative relation noted in GW359-52R is linkage between a preponderance of the genes conditioning very high percentage sucrose with a preponderance of those conditioning low weight per root. Since the genetic covariances for the other segregating populations are not statistically significant, the data from these populations do not confirm linkage as a causal agency. However, neither do they disprove linkage, as a balanced system may be involved.

A third possible explanation is pleiotropy. Again, if pleiotropy were one of the causal agencies, at least some of the genes conditioning percentage sucrose and some of them conditioning weight per root would be pleiotropic in GW359-52R and those conditioning these two characters in the other segregating populations would exhibit pleiotropy to a very limited extent. Stated another way, at least some of the physiological-genetic processes tending to produce high percentage sucrose tend to produce lower weight per root in GW359-52R; but such a relation exists to a very limited extent (if at all) in the other segregating populations. The physiological-genetic hypothesis is supported by the finding that those varieties possessing only environmental variability have statistically significant negative environmental covariances.

It should be kept in mind that all of these biological phenomena, and others not considered here, may be playing some part in producing the negative relation noted between percentage sucrose and weight per root. They may differ according to the population under consideration.

An understanding of the biological phenomena responsible for the negative relation between percentage sucrose and weight per root would be of value in conducting the breeding programs. Such knowledge should materially facilitate recombining the desirable genes for percentage sucrose with the desirable genes for weight per root. Also, an understanding of these biological phenomena should provide information as to what extent these two characters can be increased simultaneously and provide other information of value to the planning and promulgation of the breeding programs.

Evaluation of Breeding Stocks

In starting breeding programs and throughout their duration it is desirable to evaluate the different material available as breeding stocks. The methods and formulas presented in this article provide one means of so doing. Their application should provide the following information: The extent of genetic variability, the proportion in the population of the identifiable-genetically-superior individuals, genetic gains possible, and the relation between the characters the breeder is striving to recombine or increase. Such information would make possible the selection of superior stocks with which to start the breeding program, and also the production of superior breeding stocks. These superior breeding stocks can be produced in the following ways: Hybridization between identified-genetically-superior individuals selected from a given population, hybridization between genetically-superior populations, and, finally, hybridization between identifiable-genetically-superior individuals of superior populations. Also, the relative merits of these different breeding stocks so selected and produced can be evaluated by application of the methods and formulas outlined in this article.

Value to the Breeding Program

The methods and formulas are equally valuable throughout the pursuance of the breeding programs.

In asexually propagated material in which mutations may have given rise to heterogeneity, they can be used to select the individual identifiable as genetically superior. The same applies to self-fertilized crops. Also, where different methods of producing mutations are being tested the methods and formulas may be used to determine the most effective mutagenic agencies, and to determine just how effective they are in producing variability.

The application of the methods and formulas to the breeding of cross-fertilized crops will be considered in more detail. Heterosis may be of decided importance in breeding some cross-fertilized crops, and some characters may exhibit heterosis and others may not do so. In the present study, heterosis plays very little part in conditioning percentage sucrose, whereas in conditioning weight per root it plays a very decided part. This is brought out very clearly by comparing the frequency distributions within Table 1 (percentage sucrose) and those within Table 4 (weight per root). In Table 1 there is no tendency for the frequency distributions of the inbred populations to occur in the lower classes, whereas there is a decided tendency for such to be the case in Table 4 (weight per root). The selection of breeding methods in cross-fertilized crops is influenced materially by whether or not heterosis plays a decided part in conditioning the character being studied.

For those characters not exhibiting heterosis, mass selection within the cross-fertilized crops would be expected to bring about an increase in the desirable trait. In the mass selection program genetically-superior individuals can be identified by applying the methods and formulas presented in this article. The objective is to obtain as high a concentration as possible of those genes tending to increase the expression of the desirable character and those having favorable interallelic interactions.

For those characters exhibiting heterosis, the methods of breeding most generally employed are designed to utilize general combining ability, spe-

cific combining ability, or both. The polycross (Tysdal, Kiesselbach, and Westover, 23) is most commonly used to take advantage of general combining ability and the recurrent selection (Hull, 7) and reciprocal recurrent selection methods (Comstock, Robinson, and Harvey, 2) are designed to take advantage of both general and specific combining ability. The inbred line method of breeding followed by hybridization is designed to take advantage of specific combining ability. For the polycross and recurrent selection methods the different cycles of selection should be tested for proportion of identifiable-genetically-superior individuals and for genetic gains possible. The original breeding stocks and those varieties and strains that the breeding program is designed to replace should be included in these tests for purposes of comparison. Such a test should furnish information as to the progress being made and the advisability of continuing cycles of crossing followed by progeny testing and selection.

For the reciprocal recurrent selection method, the testing of the two sources of the different cycles for proportion of genetically-superior individuals and for genetic gains possible in comparison with the original material provides some information as to the desirability of continuing selection in further cycles. The inclusion of strains and varieties that the breeding program is designed to replace would serve to evaluate the two sources as possible synthetic varieties. The analysis of the frequency distribution of the hybrid between the two sources by methods and formulas given in this article would evaluate the progress being made (if any) as well as provide information concerning the desirability of continuing the hybridization, progeny testing, and selection cycles. If the proportion of individuals identifiable as genetically superior is low or if the genetically-superior individuals are inferior to those of other populations, further cycles of breeding would be of questionable value.

Inbred lines produced from one source of the reciprocal recurrent selection program should combine well with those produced from the other source. The possibility of getting inbreds whose hybrids would excel the hybrid between the two sources would be shown by testing the two sources and their hybrid for proportion of genetically-superior individuals and genetic gains possible. These tests are those discussed in the immediately preceding paragraph. The number of inbred lines that it would be necessary to produce and maintain should be comparatively few, if the reciprocal recurrent selection program is successful.

Probably one of the most important functions of the methods and formulas presented in this article is to provide a means of evaluating the breeding program as it proceeds. Likewise, whether the methods and formulas accomplish the purposes for which they were designed is subject to test as the breeding programs progress. The reliability of the methods and formulas will be indicated early in the breeding programs. However, if so desired, progeny performance tests can be made to ascertain the reliability of the methods and formulas for identifying genetically-superior individuals and predicting genetic gains possible.

Experimental Design for Selecting Individuals Genetically-Superior for Both Percentage Sucrose and Weight per Root in GW359-52R

A randomized complete block experimental design for selecting individuals genetically-superior for both percentage sucrose and weight per root in sugar beets is given in Table 10. The number of plants per culture is 24 and as can be seen from Table 10 the number of entries is 12 and the number of replications is 100. This makes a total of 28,800 plants in the experiment.

Table 10.—Randomized Complete Block Design for Selecting Individuals in GW359-52R Genetically Superior as Regards Percentage Sucrose and Weight Per Root.

Population	Entry Number	Replication and Culture Number						
		1	2	3	.	.	.	100
F_1 hybrid	1	6	17	28	.	.	.	1200
Inbred	2	7	13	26	.	.	.	1192
GW359-52R	3	2	21	34	.	.	.	1199
GW359-52R	4	1	18	31	.	.	.	1194
GW359-52R	5	11	24	33	.	.	.	1189
GW359-52R	6	9	19	35	.	.	.	1198
GW359-52R	7	4	16	25	.	.	.	1191
GW359-52R	8	8	15	32	.	.	.	1195
GW359-52R	9	12	14	27	.	.	.	1196
GW359-52R	10	3	20	29	.	.	.	1190
GW359-52R	11	10	22	30	.	.	.	1193
GW359-52R	12	5	23	36	.	.	.	1197

From Table 7 it can be seen that 2.3 percent of the GW359-52R population was identified as genetically superior for weight per root. The number of GW359-52R plants in the experiment outlined in table 10 is 24,000. Of this number 552 plants (2.3 percent of 24,000) would be expected to be genetically superior for weight per root. On the basis of independence also 2.3 percent of these plants should be identifiable as genetically superior for percentage sucrose. Hence, probably approximately 12 plants should be identifiable as genetically superior for both characters. The data show that the two characters are not independent and that the relation between them is negative. Hence, considerably less than 12 plants can be identified as genetically superior for both characters. An examination of Table 7 shows that only 0.2 percent of the individuals identifiable as genetically superior for percentage sucrose fell beyond the average for weight per root. Then, it is doubtful if even one of these 552 individuals, identifiable as genetically superior for weight per root, could be identified as genetically superior for percentage sucrose. It seems that individuals identifiable as genetically superior for both characters cannot be selected in one growing season from a population of 24,000 plants.

Another approach is to save ten percent of the roots having the greatest weight, analyze these for percentage sucrose, and save those individuals that can be identified as genetically superior for this latter character. Then, again select from this 10 percent another group which can be identified as genetically superior for weight per root and which have the highest percentages of sucrose. If among these 2,400 heavier beets any do occur which are identifiable as genetically superior for both characters, they should be saved as such and grown in a crossing plot by themselves. The other two groups should be grown in another crossing plot. In both crossing plots the seed should be saved on an individual plant basis. Asexual propagations should be made from all of the beets selected for parents and grown in the crossing plots mentioned in this paragraph. The progeny from both crossing plots should be tested in an experiment with GW359-52R to determine if any improvement has occurred in weight per root and percentage sucrose. If so, the asexually propagated roots of these progenies should be used for further breeding purposes.

When crossing the above roots selected from the experiment outlined in Table 10, provisions should be made to test for both general and specific combining ability. This can be done by cutting each root into four parts and planting two cuttings from each selection in a polycross breeding plot. In the second crossing plot composed of the other two quarters of each beet the reciprocal recurrent selection method of breeding should be employed. Those roots in which identifiable-genetically-superior individuals for percentage sucrose had been selected would be used as one source and those roots in which identifiable-genetically-superior individuals for weight of root had been selected would be used as the other source. The mechanics of the procedure outlined above have been solved and the pertinent information is being prepared for publication by Powers, Finkner, and Doxtator.

Summary

The principles and formulas developed in connection with the partitioning of variance into its components and in connection with the partitioning method of genetic analysis are applied to the identification of genetically-superior individuals of populations and to the predicting of genetic gains possible by application of suitable breeding methods within these populations.

The identification of genetically-superior individuals and prediction of the genetic gains involve analyses of the population frequency distributions.

The method of identifying genetically-superior individuals by use of the formula $z = \bar{x} + s_d x'$ is given in some detail and is illustrated. The methods of determining the values of p and z are given and are illustrated.

The methods employed identify only a portion of the genetically-superior individuals in any given population, probably a minor portion.

The percentage of identified-genetically-superior individuals listed in the fourth column of Table 3 provide a basis of predicting genetic gains.

The identified-genetically-superior individuals for any given population are members of a sub-group frequency distribution having a mean larger than that of the population in which they occur.

Predicting genetic gains involves estimating the means of these subgroup frequency distributions of which the genetically-superior individuals are members. The formula employed is $y = z - s_d x'$. Its application is illustrated.

The identification of genetically-superior individuals for percentage sucrose and weight per root simultaneously is considered.

The study of percentage sucrose and weight per root simultaneously involves the analysis of the frequency distribution for both characters.

The genetic covariance for percentage sucrose and weight per root was found to be highly statistically significant and negative for GW359-52R. For the other segregating populations the genetic covariances are negative but taken individually none are statistically significant. The biological phenomena that might be responsible for this genetic relation between the two characters are discussed.

The application of the identification of genetically-superior individuals and prediction of genetic gains to the evaluation of breeding stocks, and to the promulgation and evaluation of the breeding program, is given. In the discussion, application to the following breeding methods is stressed: Mass selection, polycross, recurrent selection, reciprocal recurrent selection, and production and hybridization of inbred lines.

An experimental design for selecting individuals genetically-superior for both percentage sucrose and weight per root in GW359-52R and its use on the basis of the findings reported herein are discussed.

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A Cytological Study of F_1 Hybrids Between Swiss Chard and Beta Webbiana[1]

HELEN SAVITSKY[2] AND JOHN O. GASKILL[3]

Introduction

The three species comprising the section *Patellares* Tr. of the genus *Beta* L. (26)[4], *B. procumbens* Chr. Sm. (13, 21, 22), *B. webbiana* Moq. (1, 5, 22), and *B. patellaris* Moq. (22) are considered as potential sources of certain valuable characters not known to occur in the sugar beet (*B. vulgaris* L.). Of special importance among those characters are high resistance to leaf spot (*Cercospora beticola* Sacc.) and to sugar beet nematode (*Heterodera schachtii* Schmidt) (7, 12, 27).

Attempts to transfer genes from the section *Patellares* directly to the sugar beet have failed consistently, the F_1 hybrids usually dying while very small seedlings (27). Recent reports by two workers have shown that viable hybrids can be obtained rather readily from matings of *B. procumbens* and *B. webbiana* with Swiss chard (*B. vulgaris cicla* L.) (19) and with certain wild species of the section *Vulgares* T[2]. (9, 23). Since Swiss chard and the latter wild species are compatible with sugar beets, it is hoped that such material may be made to serve as a bridge for transfer of desirable genes from the section *Patellares* to the sugar beet. Interest in the possibility of transferring nematode resistance to the sugar beet by such means has been stimulated further by preliminary evidence indicating that the resistance of *B. procumbens* and *B. webbiana* was passed on to F_1 hybrids resulting from matings of those species with highly susceptible Swiss chard (9).

In general the F_1 hybrids, Swiss chard X *B. procumbens* and Swiss chard X *B. webbiana*, have been sterile, and the investigations reported in this paper were undertaken in order to study the causes and to search for a possible solution. F_1 plants, Swiss chard ♀ X *B. webbiana*, like those previously described (9), were grown at Fort Collins, Colorado, as the source of material, and the cytological studies were made by the senior author at Salt Lake City, Utah.

Materials and Methods

Young floral axes of F_1 hybrid plants were fixed in chrom-acetoformol, dehydrated, and sectioned. Slides were stained by iron-hematoxylin. The samples were taken from eight F_1 hybrid plants, but the necessary meiotic stages were found in only five plants. Camera lucida drawings and photomicrographs are presented.

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[1] Report of a study made by the Field Crops Research Branch, Agricultural Research Service, U. S. Dept. of Agriculture, and the Beet Sugar Development Foundation, in cooperation with the Botany and Plant Pathology Section, Colorado Agricultural Experiment Station. This paper has been approved by the Station Director as Scientific Series Article No. 478.

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[4] Numbers in parentheses refer to literature cited.

Experimental Results

The number of chromosomes was previously studied in the species of section Patellares and the results obtained were reported by Helen Savitsky at the meeting of the Beet Sugar Development Foundation in January 1953 (25).

The number of chromosomes in *B. procumbens* and in *B. webbiana* had not been previously established[5]. *B. patellaris*, according to Bleier (4), is a diploid species ($2n = 18$). Samples of these three species from different origins were used by H. Savitsky for chromosome study. *B. procumbens* and *B. webbiana* appeared to be diploid species, with 18 chromosomes in mitotic cells and 9 bivalents in meiosis. But the plants of *B. patellaris* in all samples were tetraploid. They had 36 chromosomes in mitosis and 18 bivalents in meiosis.

The F_1 hybrids between *B. vulgaris* ($n = 9$) and *B. webbiana* ($n = 9$) all showed 18 chromosomes in the early meiotic stages. The early prophase stages in the meiosis of F_1 hybrids developed as usual in diploid beets. At the leptotene stage, the chromatin threads were freely disposed in the nucleus. Some of them were intertwisted and thickened at certain points. At the zygotene, the spiralization of the chromatin threads was more frequent and sometimes doubleness of the threads or segments of threads was observed (Figure 1). Contraction in synapsis showed the usual picture: Chromatin threads were gathered in a ball at one side of the nucleolus so that only the ends of the threads could be seen sometimes outside of the ball (Figure 2).

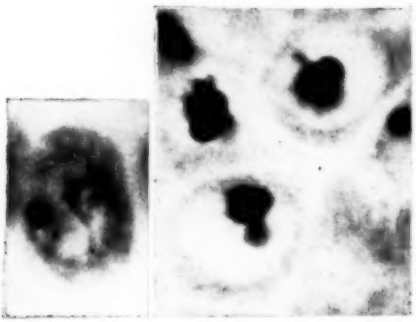

Figure 1. **Figure 2.**

Figure 1.—Zygotene in P.M.C. X 2318. Figure 2.—Synapsis in P.M. Cells X 2098.

The pairing of chromosomes was studied at diakinesis where the primary spiralization of chromosomes in early prophase could not obscure the picture. The ability of chromosomes to pair was different in different pollen mother cells (P.M.C.). Some nuclei showed typical asyndesis. They

[5] The number of chromosones for B. procumbers ($2n = 18$) was reported in Sveclovodstvo 1:6. 19, 1940. But Russian collections of wild *Beta* species did not include *B. procumbens*, therefore, this species could not be studied in Russia. The number of chromosomes in *Beta* species, known at that time in Russia, were reported by H. Savitsky in the same book (Chapter "Karyology of Genus *Beta*" p. 516). The species *B. procumbens* wasnot mentioned there.

contained 18 unpaired univalents which were scattered over the nuclei (Figure 3). In other P.M.C. from one to five paired chromosomes were observed. The distribution of bivalents is shown in Table 1.

Table 1.—Distribution of Bivalents in Pollen Mother Cells of F_1 Hybrids

Number of bivalents	0	1	2	3	4	5	Total
Number of P.M.C.	2	3	11	15	13	6	50
Percent of P.M.C. with corresponding number of bivalents	4	6	22	30	26	12	

Figure 3.

Figure 4.

Figure 3.—Asyndetic nucleus with 18^{I} X 3255. Figure 4.—Nucleus with 3^{II} and 12^{I} X 3255.

Three and four bivalents were observed in the majority of P.M.C. The cases with one or five bivalents were found rather seldom. The same concerns P.M.C. in which the pairing of chromosomes was absent. Nuclei with 18 univalents and with three bivalents are shown in Figures 3 and 4. All chromosomes at diakinesis were split lengthwise, so that the four chromatids in paired chromosomes and the two chromatids in univalents could be distinguished. The univalents usually had the shape of rods; seldom were they contracted like droplets. The bivalents were distinguished by their larger size, by the number of chromatids, and by the chiasmata connecting them. The shape of bivalents was typical for *Beta* in diakinesis. The majority of them had one chiasma located close to one end of the chromosomes, which could be called a "sub-terminal" chiasma (Figure 5—c, d, e, f, g, j, l). The majority of chiasmata was symmetrical. Quite frequently the bivalents showed one or two interstitial chiasmata (Figure 5-a). The interstitial chiasmata were very often observed in cross-shaped bivalents (Figure 5—i, n). Sometimes bivalents had one interstitial chiasma and one terminal chiasma (Figure 5-k). The terminal chiasmata were seldom observed (Figure 5-h). The chiasmata frequency was estimated at 3.04 per P.M.C. In some P.M.C., fragmentation of chromosomes could be observed (Figure 6). Several chromosomes in some nuclei showed the accentuated constrictions, and the arms of such chromosomes lay wide apart. Two chromatids, more or less

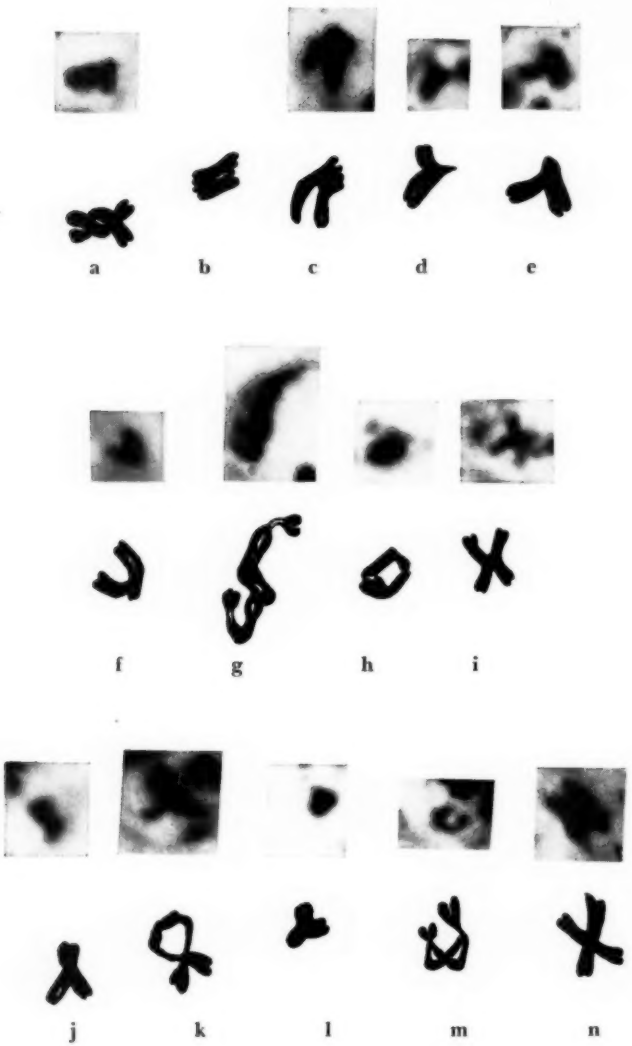

Figure 5.—The types of bivalents; bivalents with:
1 "sub-terminal" chiasma—c, d, e, f, g, j, l
1 interstitial chiasma—i, n
2 interstitial chiasmata—a, m
2 terminal chiasmata—h
1 termial and 1 interstitial chiasmata—k X 3890

separated, were clearly visible in the majority of arms. The wide constrictions were evidently due to unspiralized understained (thin) heterochromatin threads. If the breakage occurs in the elongated constriction region the chromosome will be divided into two fragments. The diakinetic nuclein containing fragments were observed in the hybrids studied. Figure 7 shows one of such nuclei containing 18 univalents and one fragment.

Figure 6.

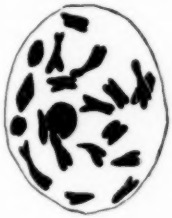

Figure 7.

Figure 6.—Fragmentation of chromosomes X 3528. Figure 7.—Nucleus with 18^{1} + 1 fragment X 3255.

At the first metaphase the chromosomes were crowded at the equatorial plate, but the contraction of the chromosomes and their orientation on the spindle was not so complete as in *B. vulgaris* or in other *Beta* species. In *B. vulgaris* the first metaphasic plate consists of small, nearly round bivalents. In F_1 hybrids many chromosomes represented shortened rods, and the ends of chromosomes were often directed to the outside of the plate. The late stages of the first meiotic division in F_1 hybrids showed many specific peculiarities deviating from the normal type of meiosis in diploids. In diploid *Beta* species the chromosomes move toward the poles in the first anaphase (IA), simultaneously, forming two narrow strips of chromosomes between the equator of the spindle and the poles. For the F_1 hybrids it was typical that the chromosomes in the first anaphase were scattered over the whole spindle. This was caused by the random orientation, non-simultaneous moving apart of the chromosomes, the majority of which were univalents, and also by the lagging of some chromosomes. Figure 8 shows a typical view of the first anaphase where some chromosomes have reached the poles while others are lying in different parts of the spindle between two poles. The distribution of chromosomes at different poles was very irregular. The total number of chromosomes lying on the spindle often exceeded 18, the number of chromosomes in the first anaphase usually observed in normal meiosis of a diploid.

In F_1 hybrids the number of chromosomes in the first bipolar anaphase varied from 18 to 28. This means that some univalents divided in the first meiotic division and others proceeded to the poles undivided.

In several P.M.C some large chromosomes could be observed at the first anaphase. Sometimes these large chromosomes consisted of four chromatids resembling in size and shape the diakinetic bivalents. In two cases there was no doubt that such large chromosomes represented bivalents.

Figure 8.

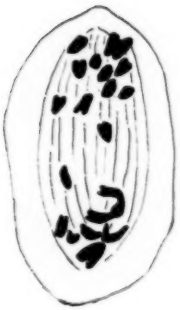

Figure 9.

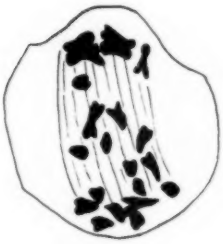

Figure 10.

Figure 8.—1 bipolar anaphase with 23 chromosomes X 3500. Figure 9.—1 anaphase nondisjunction of a bivalent X 3500. Figure 10.—1 anaphase with two bivalents at the pole X 3500.

Figure 9 shows nondisjunction of a bivalent. The bivalent is situated on the spindle near one pole and consists of two chromosomes connected by "subterminal" chiasma. Both partners of bivalents showed split ends indicating that they consisted of two chromatids. In Figure 10, two bivalents were distinguished from all other chromosomes by their larger size. At certain positions the split ends of one partner in each chromosome could be seen, which, besides their size, confirmed the fact that they were bivalents. The bivalents observed at the anaphase resembled in size (they were a little smaller) and appearance the bivalents seen in diakinesis, that is, contraction at the first metaphase (IM) was not strong enough. Nondisjunction was frequently observed by Goodspeed in X-rayed *Nicotiana tabacum* (10).

The interkinetic nuclei also sometimes contained bivalents which have been previously observed at the IA. Figure 11 shows an interkinetic nucleus with a large bivalent. One end of the bivalent had two splits, showing that it consisted of four chromatids. The curvature of the spindle was observed in a few cells.

Occasionally, during interkinesis, P.M.C. with large, non-reduced nuclei were found among binuclear P.M.C. The number of chromosomes in such nuclei equaled 18 (Figure 12). Such nuclei may give rise to a diploid pollen grain containing one genome of *B. vulgaris* and one of *B. webbiana*. The case is analogous to *Raphano-brassica* hybrids (14) and some others.

The first amphidiploid plant reported in the genus *Beta* was obtained by Zossimovitch (28, 29) after hybridization of *B. lomatogona* (n = 9) to *B. corolliflora* (n = 18). When non-reduced gametes of F_1 hybrids (n = 27) united during fertilization an amphidiploid plant arose which contained 54 chromosomes in somatic cells and resembled very much in its appearance the hexaploid species *B. trigyna*.

Figure 11.

Figure 12.

Figure 11.—Two interkinetic nuclei with 11 and 13 chromosomes; one nucleus contains a bivalent X 3500. Figure 12.—Non-reduced nucleus with 18 chromosomes X 3255.

An important deviation from the normal meiotic division in diploids was the formation of multipolar spindles at the IA of F_1 hybrids. In many P.M.C. the spindle developed three, four, or more poles. In most cases the multipolar spindle developed three or four poles which were connected by the continuous fibres, forming one common spindle. In the cells with a tri-polar spindle, the chromosomes proceeded at IA to three different poles and three nuclei were formed. Sometimes these nuclei were of approximately the same size (Figure 13), but the number of chromosomes which reached their respective poles varied in some degree. In other cases the number of chromosomes at different poles and the size of nuclei varied considerably (Figure 14). In the four-polar spindles, sometimes four symmetrical poles were formed (Figure 15), the chromosomes were distributed

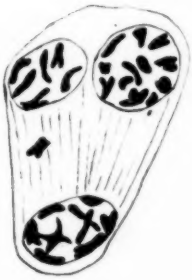

Figure 13.

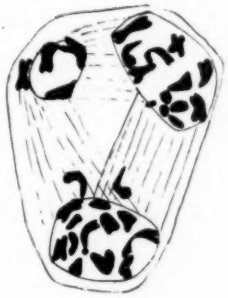

Figure 14.

Figures 13 and 14.—Late multipolar anaphase with three poles X 3500.

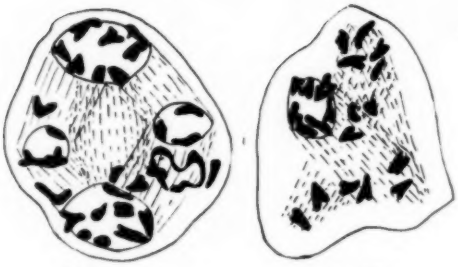

Figure 15. Figure 16.

Figures 15 and 16.—Late multipolar anaphase with four symetrical poles X 3500.

between poles very irregularly, and many of them remained on the spindle outside of the forming nuclei. Figure 16 shows the distribution of chromosomes between the poles of a four-polar spindle in another P.M.C. One group of chromosomes reached the pole, and the nucleus was already formed, while other chromosomes were on the way to the other poles.

In many P.M.C. the shape of the spindle was asymmetrical. In some cases three poles were found at one end of the spindle and the fourth pole at the opposite end (Figure 17). Sometimes the spindle formed a triangle (Figure 18), and the fourth or fifth pole was at the side on another level and the chromosomes which are marked only by their contour lines were still moving forward toward the pole. The two nuclei lying side by side could be considered either as nuclei formed at the third and fourth poles, or one of them could be a micro nucleus derived from the group of chromosomes which remained on the spindle. In some cells there were five, six, or seven poles and the chromosomes were distributed among them (Figure 19). The distribution of chromosomes in P.M.C. with a multipolar spindle was much more irregular than in the cells with a bipolar spindle and the size of formed nuclei varied considerably.

The activity of chromosomal fibers from different poles was not similar. In many P.M.C. the majority of chromosomes moved to one or two poles and only a few reached the other two poles (Figures 14, 15, 17). But the activity of the multipolar spindle in general was much higher than the activity of the bipolar spindle. More chromosomes were moving apart and reached poles than in P.M.C. with a bipolar spindle. The number of chromosomes in P.M.C. with bipolar anaphase varied from 18 to 28; the great part of the cells contained from 18 to 24 chromosomes (Table 2). In the P.M.C. with multipolar anaphase, more chromosomes were observed. The majority of these cells contained from 24 to 29 chromosomes. In two cases cells with 32 and 36 chromosomes were found. At the same time more nuclei were formed in the P.M.C. with a multipolar spindle, but they contained fewer chromosomes than the interkinetic nuclei after bipolar anaphase (Table 3). The number of chromosomes in the nuclei after bipolar anaphase varied from eight to fourteen. The nuclei after multipolar anaphase contained from one to eighteen chromosomes. Many of them had three, four, five, or six chromosomes; in the majority of these nuclei the

number of chromosomes was lower than nine, although the number of nuclei with nine chromosomes was about the same when compared with bipolar anaphase. More lagging chromosomes were observed at multipolar anaphase than in bipolar. More univalents were oriented on the multipolar spindle than on the bipolar—therefore, more chances existed for some univalents to be oriented later at the late metaphase or even at anaphase. The late-oriented chromosomes may not have time to complete their "polarization" toward two different poles or some of them will not be able to reach the poles until the nuclei are formed and they remain as laggards

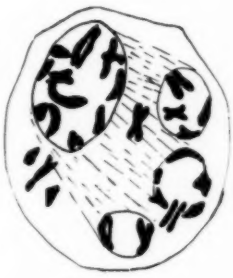

Figure 17.

Figure 18.

Figure 19.

Figure 17.—Late multipolar anaphase with four asymmetrical poles X 3500. Figures 18 and 19.—Late multipolar anaphase with more than four poles X 3500.

Table 2.—Number of Chromosomes in Pollen Mother Cells with Bi-or Multipolar Spindle in the First Anaphase.

	P.M.C. With Number of Chromosomes as follows:																	
	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36
P.M.C. with bipolar anaphase	3	1	3	1	3	1	1	1			1								
P.M.C. with multi-polar anaphase							4	1		3		5			1				1

Table 3.—Number of Chromosomes in Nuclei Formed After Bipolar and Multipolar Anaphase.

	Nuclei with Number of Chromosomes as follows:																	
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Nuclei after bipolar anaphase								1	5	5	7	5	2	1				
Nuclei after multipolar anaphase	1	8	6	3	3	8	6	8	6	4			1	2				2

on the spindle. At any rate, three, four, or five chromosomes often were seen on the spindle at the time the nuclei were formed at the poles after multipolar anaphase instead of one or two after bipolar anaphase. The bivalents which were observed in the bipolar anaphase and sometimes in the interkinetic nuclei after bipolar anaphase never were seen on the multipolar spindle or in the nuclei derived after the multipolar anaphase. This also confirms the fact that the moving of chromosomes to the poles and the co-orientation of the bivalents were more easily completed by the multipolar spindle. The chromosome fibres extending from the poles in different directions could more easily co-orient the kinetochores of a bivalent towards two different poles, because the angle of turning of centromeric chromomeres and of the kinetochore itself may be much smaller than on the bipolar spindle.

In some multipolar spindles certain poles were situated very close to each other, so that the nuclei formed at the poles often lay side by side (Figures 18, 19). In some cells the fusion of two groups of chromosomes lying at closely situated poles could be observed. Figure 21 shows fusion of two groups of chromosomes to form one nucleus with 18 chromosomes. Figure 22 represents another similar case. An enormous nucleus of a very irregular shape and not yet completely formed lies at one pole. It also contains 18 chromosomes. The number of chromosomes in both these nuclei considerably exceeded the number usually observed in one nucleus after anaphase. Such nuclei with high chromosome numbers may originate either from the fusion of two groups of chromosomes at two closely located poles, or from partial bipolarization of the muitipolar spindle, as was observed in a *purpurea* haploid of *Nicotiana* (6).

Figure 20.

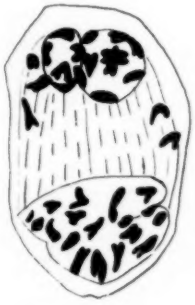

Figure 21.

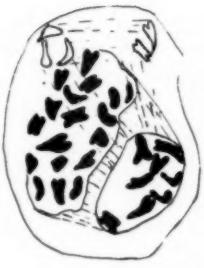

Figure 22.

Figure 20.—P.M.C. with 36 chromosomes containing a hexad formed after meiosis X 3500. Figure 21.—Fusion of two groups of chromosomes at closely located poles in multipolar anaphase X 3500. Figure 22.—Incompletely formed nucleus with 18 chromosomes derived from fusion of two groups of chromosomes; the ples with chrmosomes marked with cntour lines are at different level X 3500.

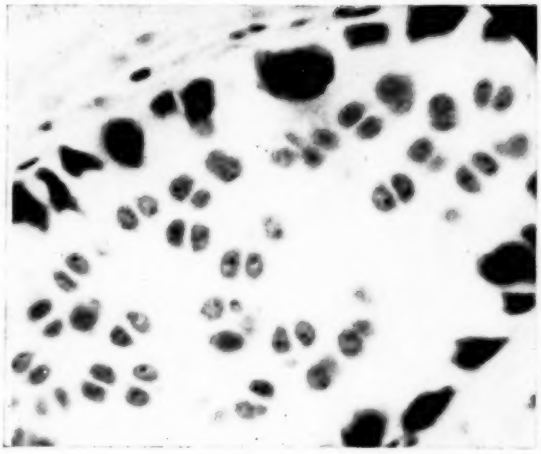

Figure 23.

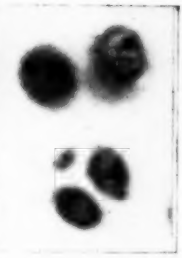

Figure 24.

Figure 23.—Locule of an anther containing almost exclusively dyads formed after 1 bipolar anaphase X 607. Figure 24.—Dyad and a dyad with a little additional cell or a triad X 1600.

The second meiotic division did not take place in F_1 hybrids. After the interkinetic nuclei were formed, the walls of pollen mother cells collapsed and the nuclei surrounded by cytoplasm were freely disposed in the locules of anthers where they gradually developed into pollen grains. The pollen mother cells which had two nuclei after bipolar anaphase produced dyads (Figure 23). If, besides the two nuclei, the micronucleus was formed, the dyads also contained a small third cell (Figure 24). The dyads were not rare; in some locules of anthers the majority of pollen mother cells produced dyads almost exclusively (Figure 23). In other locules, besides dyads, triads, tetrads, pentrads, etc., were formed which developed after multipolar anaphas (Figure 25). Both types of the anaphase (bipolar and multipolar) occurred with about the same frequency, and often the tetrad-stage in pollen mother cells lying side by side showed different types of anaphas (Figure 26).

There were great variations in the size of nuclei and cells in tetrads, pentads, etc., as well as in the size of pollen grains derived from them (Figure 27). Very small pollen grains developed no nucleoli and were abortive just after their formation. In open flowers some anthers dehisced and some did not. In certain flowers the anthers stood on outstretched filaments for a longer than usual period of time without dehiscing. The pollen grains in the anthers of open flowers were yellow, with both intine and extine being developed. Some of them were round, but most had irregular shapes, sometimes they were crescent in shape, resembling pollen grains of the synaptic forms in *Allium amplectens* (16). The size of pollen grains varied from 6μ to 26μ, the majority of them equalling 15μ. In diploid sugar

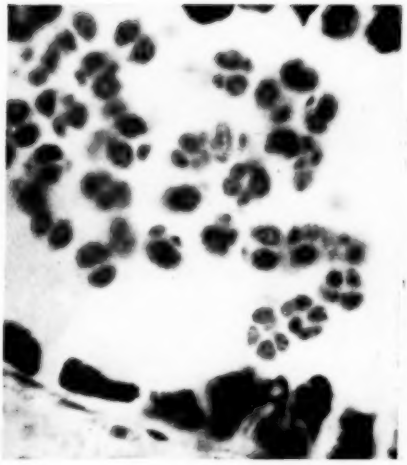

Figure 25.

Figure 26.

Figure 25.—Locule of an anther containing tetrads, pentads, hexads, etc. formed after 1 multipolar anaphase X 607. Figure 26.—Two dyads, tetrad and a pentad lying side by side X 1600.

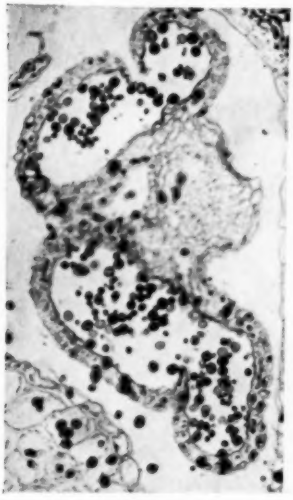

Figure 27.

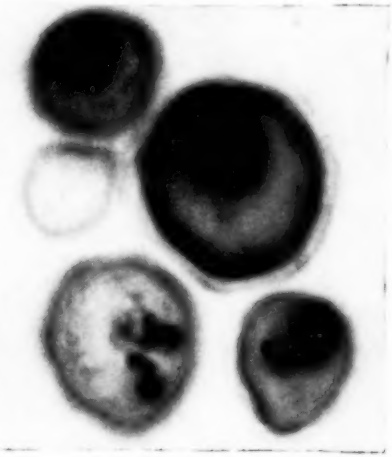

Figure 28.

Figure 27.—Sectioned matured anther containing pollen grains of different sixes X n 109. Figure 28.—Sectioned pollen grains from an opened flower; empty pollen grain and pollen grains with one an dtwo degenerating nuclei X 2000.

beets the size of pollen grains usually varies from 19μ to 21μ. Examination of sectioned pollen grains from matured buds and open flowers showed that 74.7 percent of them still remained at the one-nucleus stage instead of the three-nuclei stage which is normal at this period of development (Table 4 and Figure 28). In many mononuclear microspores the nuclei were dead (dark-stained or destroyed) and 21.8 percent of pollen grains were empty without plasma and nuclei. Only three percent of the microspores developed a tube nucleus and a generative nucleus. But, in spite of the picture of general disorganization, 0.5 percent contained three nuclei, two sperm nuclei and a tube nucleus. Some of these pollen grains may be functional.

Table 4.—Pollen Grains in the Anthers of Open Flowers.

	Empty	With One Nucleus	With Generative and Tube Nuclei	With 2-Sperm Nuclei and Tube Nucleus	Total
Number of pollen grains	218	747	30	5	1000
Percent	21.8	74.7	3.0	0.5	100

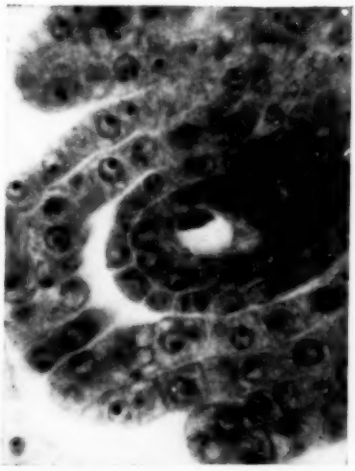

Figure 29.—Synapsis in megaspore mother cell X 833.

Discussion

In remote interspecies hybridization, such as a hybridization of *B. vulgaris* with *B. webbiana*, the absence of chromosome association in F_1 hybrids could be regarded as normal. However, cytological study showed that in most P.M.C. of F_1 hybrids, three or four pairs of chromosomes were observed.

This appearance must be considered as a positive one. The final purpose of such hybridization consists of transferring desirable genes, such as those providing for resistance to nematodes, from species of the section *Patellares* (including *B. webbiana*) to the species *B vulgaris*.

If the chromosomes of *B. vulgaris* and of species of the section *Patellares* could not associate, almost no chance would exist of obtaining nematode-resistant sugar beets by use of interspecies hybridization. Although diploid hybrids between these species were sterile, and preferential association between chromosomes of the same species must be expected in tetraploid hybrids, yet the ability of chromosomes of *B. vulgaris* to associate with chromosomes of *B. webbiana* is a very hopeful sign that chromatin transfer between these diverse species can be expected, provided fertile hybrids are obtained.

The shape of bivalents in F_1 hybrids closely resembled the shape of bivalents usually observed in *Beta* species. Therefore, it could be assumed that the chiasmata frequency in the bivalents of hybrids did not greatly differ from the chiasmata frequencies in the bivalents of *Beta* species in diakinesis.

It may be suspected that part of the bivalents in F_1 hybrids may result from association of chromosomes within a baploid set. Some haploids (or monoploids), such as haploids of *Nicotiana tabacum* (6), of *Datura stramonium* (2, 3), etc., did not show chromosome pairing, but in other haploid plants *Antirrhinum* (11), *Zea mays* (20), *Secale cereale* (17), *sorghum* (15, 8), and *B. vulgaris* (18), the pairing of chromosomes were observed. A. Levan (18) described the pairing of chromosomes in a haploid sugar beet plant, but the pairing in haploid rye, sugar beets, and some other species is mainly a spiralization pairing through early prophase stages and gradually disappears during diplotene-diakinesis.

The great distinction, according to Levan, between pairing in diploids and haploids is that the haploid gives rise to only a few chiasmata and these chiasmata may have been caused by purely chance factors and were not formed between homologous segments. The chiasmata frequency in sugar beet haploid plants varied for different fixations from about 0.5 to 0.1 per cell. In the F_1 hybrids studied the chiasmata remained until the first metaphase and their frequency was much higher; it was evaluated at 3.04 per P.M.C. The chiasmata frequency might be reduced but little during the first metaphase. It is impossible to prove that not one bivalent arose in F_1 hybrids from pairing of chromosomes within a haploid set, but the chiasmata frequencies and the late meiotic stages, where they were observed, indicated that it is much more probable that the pairing took place between homologous chromosomes of *B. vulgaris* and *B. webbiana*. In many interspecies hybrids the pairing between chromosomes of two parental species was usually observed.

Among different abnormalities in meiosis of F_1 hybrids the defects of the spindle could be mentioned. Nondisjunction of bivalents in bipolar anaphase could be explained by insufficient activity of the poleward forces of the spindle. The arrangement of chromosomes at the equatorial plate at the first metaphase and the moving of chromosomes toward the poles

depend upon the poleward forces (the a-factor) of the spindle and upon the orientation of kinetochores of chromosomes. Nondisjunction of bivalents could occur when the kinetochores of such bivalents were no co-oriented on the equatorial plate because one chromosome of the pair was not attached to the chromosome fibre, or because of the insufficient activity of the pulling forces (poleward attraction) of one of the poles.

Development of multipolar spindles with three or four or sometimes a higher number of poles is also a deviation form the normal type of division. But, in spite of this, the multipolar spindle functioned more efficiently than the bipolar. More chromosomes were moved apart and transported to the poles in multipolar anaphase. It is probable that more chromosomal fibres crossing the nucleus in different directions were developed in P.M.C. with a multipolar spindle resulting in more effective movement of the chromosomes to the poles. Higher activity of the multipolar spindle can be explained on the basis of the hypothesis of orientation by pulling (24). The majority of chromosomes in the P.M.C. of F_1 hybrids are univalents. The kinetochores of the univalents are oriented at random in various directions. On the multipolar spindle the poleward attraction (the a-factor) working on the kinetochores of the univalents can more easily arrange them in various positions on the spindle. The chromosomal fibres extending from poles in different directions can more easily meet the centromeric chromomeres of the univalents (which often show an angular separation), arrange the univalents on the spindle, and lead to their "polarization." As a result more univalents may divide in the multipolar anaphase than in the bipolar.

The negative consequence of the activity of a multipolar spindle was the formation of a higher number of nuclei, each containing a lower number of chromosomes. This led to the formation of a large quantity of nonviable pollen grains. The total number of chromosomes in P.M.C. of F_1 hybrids was lower after meiosis than in the normal diploid beet. Thirty-six chromosomes were observed in only one cell. In the majority of P.M.C. the highest number of chromosomes varied from 27 to 32. Evidently not all univalents were divided during the first division. The formation of dyads in these hybrids was due to the failure of the second meiotic division.

A cytological study of F_1 hybrids between Swiss chard and *B. webbiana* revealed the causes of their sterility. Because of the high sterility of the gametes in these F_1 hybrids, there were but few chances to obtain the progeny from intercrosses between F_1 plants. But, even so, the diploid hybrids between *B. vulgaris* and species of section *Patellares* are not completely hopeless. The pollen grains developed from the nuclei with a low chromosome number such as 2, 3, 4, 5, etc., will be nonfunctional. It is quite doubtful that all haploid pollen grains (with nine chromosomes) and the diploid pollen grains (with 18 chromosomes) derived from the union of two groups of chromosomes in anaphase will be completely viable. Their viability will depend upon cooperation of different chromosomes obtained from both species, *B. webbiana* and *B. vulgaris*, and their physiological activity, therefore, may be limited.

The non-reduced nuclei (with 18 chromosomes) will produce viable pollen grains. If two non-reduced gametes of F_1 hybrids could meet, am-

phidiploid fertile hybrid plants could develop. Only a few non-reduced gametes were observed in F_1 hybrids between Swiss chard and *B. webbiana*. To increase the possibility of the formation of non-reduced gametes, it might be reasonable to involve in crosses species of the section *Patellares*, and the diverse races of *B. vulgaris*. On the other hand, the chances of development of amphidiploid plants will become higher if more F_1 hybrid plants are produced. It may also be desirable to grow a clone of a self-sterile, tetraploid sugar beet, together with an isolated group of F_1 hybrids. In this way the non-reduced gametes of hybrids may be more likely to meet the diploid female or male gametes of the sugar beet and produce viable tetraploid offspring, with three genomes of *B vulgaris* and one genome of *B. webbiana* or *B. procumbens*. Another way, which seems more promising, consists of the production of tetraploid hybrids either by obtaining amphi-diploid plants from colchicine treatment of diploid F_1 hybrids, or by crosses of tetraploid sugar beets with tetraploid plants from the species of the section *Patellares*.

The F_1 tetraploid hybrids will represent amphidiploids and will be fertile or semi-fertile. Their intercrosses and backcrosses to tetraploid sugar beets will produce many new combinations of chromosomes of parental species as well as the interchanges between segments of chromosomes. At the same time, a number of chromosomes higher than in the diploid hybrids will provide for a broader genetic base and higher viability of the offspring.

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Effect of Moisture and Fertilization on Emergence of Sugar Beet Seedlings[1]

F. W. SNYDER[2]

Collings (1)[3] has discussed the effect of fertilization practices on germination and emergence of field crops and has indicated that soil moisture is a key factor in the response obtained. As an aid in evaluating best field conditions for emergence of sugar beets, emergence data were obtained, in the laboratory, under controlled conditions of soil texture, moisture, and temperature; and rate, analysis, and placement of fertilizer.

Methods and Materials

Lock sandy loam top soil, obtained from a field on the Michigan State University farm at East Lansing, Michigan, was air dried, screened through a No. 20 mesh sieve, and mixed. The textural analysis was as follows: 60.4 percent sand, 26.7 percent silt ($< 50\mu$ and $> 2\mu$), and 12.9 percent clay. The moisture curve is shown in Figure 1. This soil was of medium fertility as determined by the Spurway reserve test. Moisture of the air-dried soil was determined and corrections made to give the percentage of moisture desired on an oven-dry basis (105° C.).

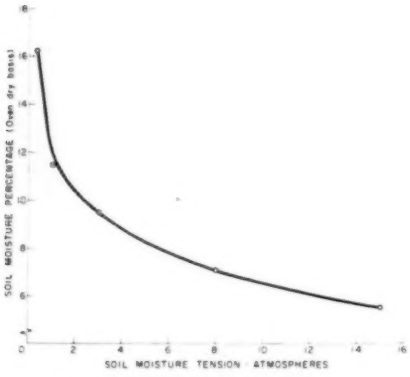

Figure 1. — Soil moisture curve for the Lock sandy loam used in this investigation.

Three hundred and forty grams of air-dried soil were placed in each plastic sandwich box (4½" x 4½" x 1⅛"). The fertilizer, broadcast or banded, was applied to the air-dried soil. In each box, eight carefully selected and sized whole seedballs of sugar beet variety US 401 were planted at a uniform depth of one-half inch. Finally, distilled water was added over the surface to give the desired soil moisture. The shallow depth of the soil, approximately seven-eighths of an inch, permitted moisture equilibrium

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[1] Cooperative investigations of the Field Crops Research Branch, Agricultural Research Service, U. S. Department of Agriculture, and the Michigan Agricultural Experiment Station. Approved for publication as Journal article No. 1881, Michigan Agricultural Experiment Station.

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[3] Numbers in parentheses refer to literature cited.

sufficiently rapid to avoid any serious difficulty. In experiments involving more than four days, distilled water was added every three to five days to replace that lost by evaporation. The plastic covers were loose enough to permit a loss of up to 0.6 percent soil moisture in a four-day period. The boxes were kept in an incubator at 20° C. ± 2° for the duration of each experiment. Counts of seedlings were made every 24 hours. Only the first seedling from each seedball was counted. Each treatment was replicated four times. The maximum duration of an experiment was 20 days.

For broadcast fertilization, the fertilizer incorporated in each box was equal to the amount that would have been broadcast over and thoroughly mixed in the top three inches of the soil of comparable surface area (1000 lbs. per 1,000,000 lbs. of soil or 0.34 gms. fertilizer per 340 gms. of air-dried soil). For band application the fertilizer was placed as evenly as possible in a band approximately a quarter-inch in width. The amount applied per lineal inch was calculated at the acre rate for 28-inch rows. Where seedballs were to be in contact with fertilizer, the band of fertilizer was applied and the seedballs placed directly on the band of fertilizer.

Results

The number of days required for 75 percent emergence with different moisture levels and rates and placement of 10-10-10 fertilizer are shown graphically in Figure 2. High soil moisture minimized the effect of quantity and placement of fertilizer. On the other hand, soil moisture in the range in which field operations are done was sufficiently limiting to delay emergence where 50 and 100 pounds of fertilizer per acre were banded in contact with the seed.

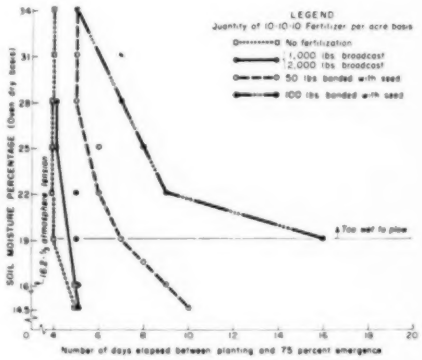

Figure 2.—Effect of soil moisture and fertilization on emergence of seedlings of sugar beet variety US 401.

The marked effect of different rates of fertilization on emergence at a given soil moisture is shown in Figure 3. Figure 4 reveals the effect of given levels of soil moisture, when the rate and placement of fertilizer were maintained constant. With reductions in soil moisture, emergence was not only delayed, but there was a substantial reduction in percentage of emergence.

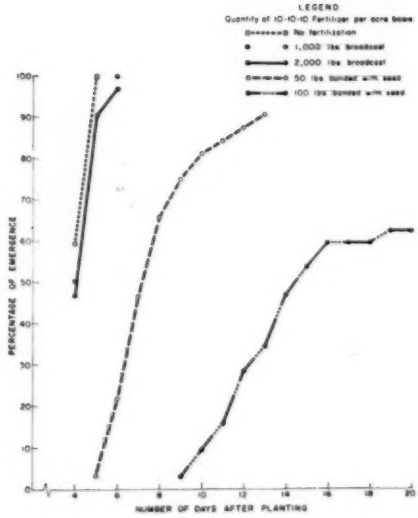

Figure 3.—Effect of fertilization on accumulated percentage emergence of seedlings of sugar beet variety US 401 at 16.2 percent soil moisture.

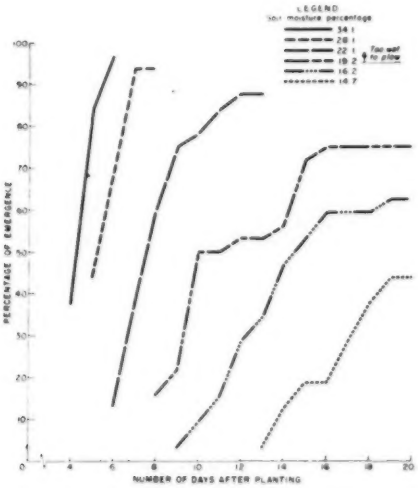

Figure 4.—Effect of soil moisture on accumulated percentage emergence of seedlings of sugar beet variety US 401. 100 pounds of 10-10-10 fertilizer per acre banded in contact with seeds.

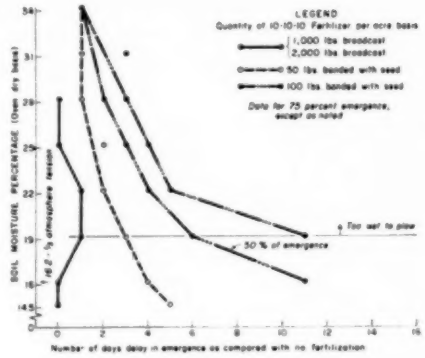
LEGEND
Quantity of 10-10-10 Fertilizer per acre basis
1,000 lbs broadcast
2,000 lbs broadcast
50 lbs. banded with seed
100 lbs. banded with seed
Data for 75 percent emergence, except as noted
Too wet to plow
50 % of emergence
SOIL MOISTURE PERCENTAGE (Oven dry basis)
Number of days delay in emergence as compared with no fertilization

Figure 5.—Delays in emergence of sugar beet seedlings of variety US 401 resulting from specific fertilization practices at given soil moisture percentages.

The extent of delay in emergence of seedlings because of a given fertilization practice may be determined by a comparison with the emergence where no fertilizer was applied. This delay has been expressed graphically in Figure 5. At 28.1 percent soil moisture, 100 pounds of 10-10-10 banded with the seed delayed emergence three days as compared with emergence of seedlings receiving no fertilization. However, at 19.2 percent soil moisture, the delay in attaining 75 percent emergence was eleven days. In an experiment at 16.2 percent soil moisture, 0-45-0 fertilizer banded in contact with the seed at a calculated rate of 50 pounds per acre delayed emergence two days as compared with no fertilization.

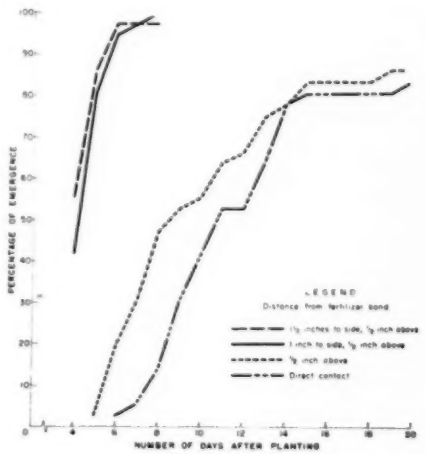

Figure 6.—Effect of distance of seeds from fertilizer band (100 pounds 10-10-10 per acre basis, 28-inch rows) on emergence of sugar beet seedlings of variety US 401. Soil moisture 16.2 percent.

To determine the effect of a soil barrier between the seed and fertilizer on emergence, six replications were used, each box containing three rows of six seeds per row placed at different distances from 10-10-10 fertilizer, banded at the rate of 100 pounds per acre. These results are shown graphically in Figure 6. Where seedballs were placed (a) in contact with fertilizer, (b) one-half inch directly above the fertilizer, or (c) one inch to the side and one-half inch above the band of fertilizer, those seeds placed at the greater distance germinated most rapidly. Seeds at the 1-inch and 1½-inch distances germinated equally well.

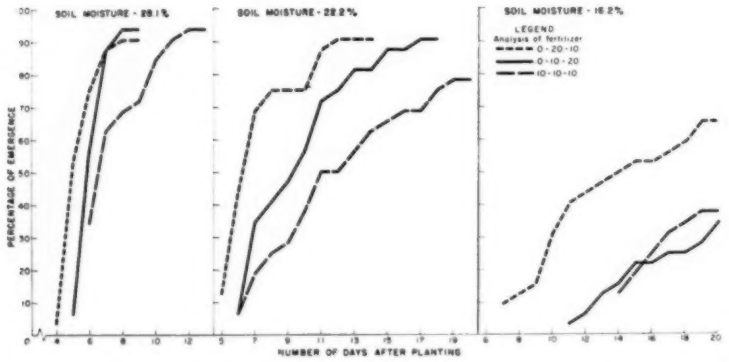

Figure 7.—Effect of 100 pounds per acre basis (28-inch rows) of different analyses of fertilizer banded in contact with the seeds of sugar beet variety US 401 on emergence.

The effects of each of three fertilizers banded in contact with the seedballs on emergence is illustrated graphically in Figure 7. The 10-10-10 analysis was a commercial mix while the 0-10-20 and 0-20-10 were compounded by hand mixing. At the same rate of application and the same soil moisture percentage, the 0-20-10 caused the least delay in emergence while the 10-10-10 caused the greatest delay. This differential delay in emergence of the seedlings appears to be linked directly with the degree of solubility of these three grades of fertilizer. The solubility data[3] were obtained as follows: One part of fertilizer was dissolved in 100 parts of distilled water for one hour. The conductance readings were then obtained on the Solu-bridge soil tester at 80° F. The specific conductance for the 0-20-10 was 380 x 10^{-5}; the 0-10-20 analysis 505 x 10^{-5}, and the 10-10-10 analysis 1000 x 10^{-5}. The high specific conductance reading of the 10-10-10 fertilizer was associated with the greatest delay in emergence.

[3] Data determined by J. C. Shickluna, Soil Science Department, Michigan State University.

Discussion

The interpretation of these results must be guided by the fact that the maximum soil moisture at which the plowing operation could be done under field conditions without serious detrimental effect upon the physical properties of the soil is approximately 19 percent. Thus, unless soil moisture is added by rain or irrigation after planting, critical delays in emergence may occur when fertilizer is placed with the seed at planting time.

The curves obtained suggest a good quantitative relationship between the effect of soil moisture and fertilization and the emergence pattern obtained. Surprisingly good prediction was possible from just one curve and a portion of another. Where fertilizer is placed with the seed, at moderate soil moisture, the data show that only relatively small differences in rate of application and placement may cause appreciable differences in rate of emergence of the sugar beet. This certainly substantiates the field observations cited by Collings (1) concerning the effect of fertilizer in contact with the seed. The delayed emergence resulting from fertilizer placed one-half inch from the seed emphasizes the danger of applying fertilizer with the seed.

The germination response is intimately controlled by rate of water uptake by the seed. This rate of uptake is decreased as the osmotic pressure of the soil solution is increased by depletion of the soil moisture, and the addition of fertilizer salts would intensify the effect.

Two practical implications of this study should be stressed. First, conservation of soil moisture during tillage operations is paramount in helping avoid delayed emergence. This suggests some form of minimum seedbed preparation. Secondly, proper placement of fertilizer may aid materially in obtaining rapid emergence and the establishment of a good stand of seedlings.

Summary

The effect of controlled soil moisture levels and specific fertilization practices on emergence of the sugar beet was determined. Plastic sandwich boxes were used to reduce moisture losses. The experiments were conducted at 20° C. ± 2°.

1. For a given fertilizer rate and placement, as the soil moisture level was decreased, emergence was progressively delayed. A substantially lower percentage of seedlings emerged at the lower moisture levels.

2. Banding 100 pounds per acre (28-inch rows) of 10-10-10 fertilizer in contact with the seed at 14.7 percent soil moisture resulted in emergence of less than 50 percent of the potential stand in twenty days.

3. Emergence was substantially delayed when seeds were placed one-half inch above the band of fertilizer as compared with seeds placed one inch to the side and one-half inch above the fertilizer.

4. At the same rate of application in contact with the seeds, 0-20-10 delayed emergence least, 0-10-20 was intermediate, and 10-10-10 delayed emergence most. This was correlated with solubility of the fertilizers.

5. It is recommended that fertilizer be placed away from the sugar beet seeds to avoid delays in emergence at the lower soil-moisture levels.

Acknowledgment

The valuable technical advice of Drs. R. L. Cook and A. E. Erickson and the use of moisture tension equipment and facilities of the Soil Science Department, Michigan State University are acknowledged with appreciation.

Reference

(1) COLLINGS, G. H. 1955. Commercial Fertilizers. McGraw-Hill Book Co., Inc., 5th Ed.

Factors Influencing Phenotypic Expression of Cytoplasmic Male Sterility in the Sugar Beet (Beta Vulgaris L.)[1]

G. J. HOGABOAM[2]

Introduction

The small, perfect flowers and indeterminate flowering habit of the sugar beet makes large-scale controlled cross-pollinations impracticable without the cumbersome use of marker genes for identification of true hybrids. Although self-sterility has been used to enforce cross- and sib-pollinations, this character complicates inbreeding procedures and does not yield all true hybrids.

Large scale production of truly hybrid seed appeared feasible with Owen's (1)[3] discovery of cytoplasmically inherited male-sterility. This discovery made it possible to develop, by repeatedly backcrossing, the male-sterile equivalent of a desired hermaphroditic plant for use as the female parent of a cross. However, Owen (1) reported partial pollen restoration through the action of nuclear genes (tentatively two dominant pollen restoring genes, X and/or Z, were assumed) in this type of cytoplasmic male-sterility. Therefore, it is also necessary to have the pollen parent of the pollen-sterile equivalent (female) line recessive for the pollen-restorer gene or genes. Such pollen-fertile parent plants, designated by Owen (2) as type O, have the cytoplasmic-genetic formula of N *xx* or N *xxzz* and owe their pollen production ability to the N (normal) cytoplasm as contrasted to the S cytoplasm of the male-sterile plants.

In the search at East Lansing, Michigan, for type O plants, many plants were found to be segregating for the pollen restorer gene or genes but no true breeding type O plants were found. The present study was undertaken to evaluate the genetic factors influencing male-sterile phenotypes. The difficulties in classifying genotypes heterozygous for the pollen-restorer gene and a new gene locus (*Sh*) enhancing the pollen-restoration ability of the X and/or Z genes without itself being a pollen restorer gene are discussed in this paper.

Experiment 1. S_1 progeny of 13H15-169.

A cytoplasmic semi-male-sterile plant, 13H15-169, considered to be heterozygous for monogermness, M^1m, (3) was selfed to study the anther types of the offspring and possible linkages between X or Z genes and M^1m genes. The progeny plants from this plant were classified for monogerm and double germ type plants and also for anther types, according to the following eight classes:

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[1] Cooperative investigation of the Crops Research Division, Agricultural Research Service, U. S. Department of Agriculture, and the Michigan Agricultural Experiment Station (Journal Article No. 1907 of Michigan Station). Part of a thesis submitted to the Faculty of the Graduate School, Michigan State University, in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

[2] Research Agronomist, Crops Research Division, Agricultural Research Service, U. S. Department of Agriculture, East Lansing, Michigan.

[3] Numbers in parentheses refer to literature cited.

Y_1—all anthers yellow and dehiscing.

Y_2—all anthers yellow, no dehiscing observed.

Y_3—most anthers yellow and dehiscing but some anthers, at least, were not definitely yellow.

Y_4—most anthers yellow, no dehiscing observed, some anthers "white."

Y_5—most anthers "white" but some anthers yellow and partially full.

Y_6—most anthers "white" but some anthers yellow and shrunken.

W_1—anthers dark and shrunken with no yellow visible or anthers a light buff color not typical of white anthers.

W_2—anthers white with no yellow coloration observed.

Table 1.—Number of Plants in Each Phenotype in the Selfed Progeny of Plant 13H15-169.

	Anther Types								
	y_1	y_2	y_3	y_4	y_5	y_6	w_1	w_2	Total
Monogerm (mm)	6	0	2	1	0	3	4	33	49
Double germ (M-)	37	2	14	4	10	3	15	53	138
Total	43	2	16	5	10	6	19	86	187

The data, Table 1, indicate that the beet 13H15-169 was heterozygous at the locus for anther color and at another locus for monogermness. Assume the cytoplasmic and genetic constitution of beet 13H15-169 for anther color and monogermness to be S XxM^1m. The Xx genotype apparently shows varying degrees of incomplete dominance since there were far too many white anthered plants in the progeny to permit an explanation that one dominant X gene will always result in a yellow anthered plant. It could be postulated that: the homozygous dominant (XX) genes in male-sterile cytoplasm (S) produce plants which have all yellow anthers; the homozygous recessive (xx) genes in S cytoplasm give all white anthers; and the heterozygous (Xx) plants have anther type classifications which range from most anthers yellow (Y_3) category through the white anther categories. Then the homozygous dominant with an all yellow anther phenotype (Y_1 and Y_2) can be tested versus the combination of all other phenotypes in a 1:3 ratio. Such a chi-square test gave a good fit, with a probability figure of 70 to 80 percent (Table 2). Another possible explanation for anther color would be a duplicate recessive epistasis ratio, in which xx is epistatic to Z, and zz is epistatic to X. Such a gene action would give a ratio of 9 yellow to 7 white. The fit of this ratio to the data is dependent upon the genotype of the W_1 anther color phenotype. If the W_1 anther color type is classed as yellow, the chi-square test gives a fit of P = 50 to 70 percent, however if the W_1 anther color is considered as actually white then the chi-square test gives a fit of P = less than 1 percent. Other data (Experiment 2) to be discussed later in this paper agree with the postulates of the first explanation of the inheritance of anther color, that XX = all yellow anthers, Xx = ranges from some plants with mostly yellow anthers to some plants with all white anthers, and xx = all white anthers.

Table 2.—Chi-Square Tests of 13H15-169 S_1 Progeny.

Class	Observed	Calculated	P Value
1:3 Segregation All Yellow Full Anthers vs. "White Anthers"			
Yellow (Y_1Y_2)	45	46.75	70-80%
"White" (Y_3-W_2)	142	140.25	
	187	187.00	
Multigermness vs. Monogermness 3:1 Segregation			
Multigerm	138	140.25	
Monogerm	49	46.75	70%
	187	187.00	
Combined Germ Type and Anther Color (Independent Inheritance)			
Yellow multi-	39	35.06	10-20%
"White" multi-	99	105.19	
Yellow mono-	6	11.69	
"White" mono-	43	35.06	
	187	187.00	
Combined Germ Type and Anther Color (Linkage in Coupling Phase with 37.5 Percent Crossover)			
Yellow multi-	39	40.18	
"White" multi-	99	100.08	
Yellow mono-	6	6.57	
"White" mono-	43	40.17	95-98%
	187	187.00	

The plant 13H15-169 was heterozygous for the monogerm character. The multigerm factor was probably the M^1 allele described by Savitsky (3) since no more than two flowers per cluster were observed on the parent plant or its progeny. The chi-square test in Table 2 indicates a P of 70 percent for one set of alleles (M^1m) controlling the monogerm segregation.

When the two factors, anther color and monogermness, were tested together in a chi-square test the P value dropped from 70 percent for each independent ratio to 10 to 20 percent for combined independent inheritance. If, however, a linkage in the coupling phase, with 37.5 percent crossover, is assumed then the P value is raised to 95 to 98 percent. Another possible explanation for the drop in P value would be pleiotropic action of the monogerm gene in recessive *mm* genotypes to give more "white" phenotypes. Further tests are needed to determine which of the theories are correct.

Experiment 2. Progeny I5-1-7E2

The first backcross progeny (I5-1-7E2) from a white anthered cytoplasmic male-sterile female and a second generation inbred male was used for a detailed study of another type. From this progeny, 128 plants were given permanent numbers to identify them in the subsequent studies. The plants were individually classified at four different dates as to anther color, fullness of anthers, and dehiscence. They were first classified in the field on June 29, 1954, when they were past full bloom. These same stecklings sent out a second growth of seed stalks as the first crop of seed was maturing. The anthers were classified again August 27, which was near the end of the second blooming period. The plants were potted in the fall and moved to the greenhouse where the third reading was made on February 24, 1955, just previous to full bloom and the fourth on March 3, 1955, as the terminal blooming period approached.

The following anther classification was used:

1_y—yellow, full, dehiscing.
2_y—yellow, full, delayed dehiscence.
3_y—anthers of earliest blooms lacking definite yellow colorations and shrunken in appearance, however in later blooms yellow partially full and dehiscing anthers appear.
4_y—same as 3_y except with delayed dehiscence.
5_y—yellow, shrunken, no dehiscence. Anthers of earliest blooms may lack definite yellow coloration.
6_w—anthers white, shrunken, sometimes darkened before blooming.

Table 3.—Random Sample of 47 Percent of the Yellow Anthered Phenotypes Giving Their Classification at Four Different Dates, Their Tendencies to Set Selfed Seed, and Genotypes Where Identified.

Pl. No.	Self Seed Set	Anther Class by Month 6 8 2 3	Pl. No.	Self Seed Set	Anther Class by Month 6 8 2 3	Pl. No.	Self Seed Set	Anther Class by Month 6 8 2 3	Identified Genotype (Table 7)
3	n	3 6 v 5	67	m	5 3 3 3	21	g	1 1 1 1	S XxShSh
5	n	5 5 5 5	75	u	3 4 1 1	51	g	1 1 v 1	S XxShSh
24	m	4 3 1 1	84	d	5 5 d d				
28	d	5 5 d d	87	u	1 1 1 1	53	g	1 1 1 1	S XxShsh
31	m	4 3 1 1	88	u	3 5 4 3	85	g	1 1 4 3	S XxShsh
35	m	2 4 6 5	93	u	5 5 5 5	98	g	1 1 1 1	S XxShsh
						113	g	1 1 1 1	S XxShsh
38	m	3 1 1 1	94	u	6 5 6 5				
42	m	1 1 1 1	102	n	1 1 1 1	12	g	5 5 3 3	S Xxshsh
45	n	6 5 5 5	109	m	1 1 1 1				
58	u	5 6 5 5	111	u	1 1 1 1				
59	m	1 3 1 1	120	m	5 6 5 5				
63	u	3 5 5 3	126	n	1 1 1 1				

n = no selfed seed set
u = selfed "seeds" but embryos undeveloped
m = good selfed seed but not enough for progeny test
g = enough good selfed seed for a progeny test
d = plant dead
v = plant not blooming when the classification was made

Of the 128 plants, 4 died after the first reading of anther type; 23 of the 128 were always judged to be 1_y, and of these, 4 set no selfed seed, 2 set seed with imperfect embryos, and the other 17 set normal selfed seed but only 10 produced enough for progeny testing; 28 of the 128 had yellow full anthers in at least one of the classifications and also fell within the categories 3_y to 6_w at least one time in the four different classifications; 17 of the 128 had at least one classification of 5_y and either a 5_y or 6_w at other times, 10 of these were 5_y at all classifications; 56 of the 128 were never observed to be in a category other than 6_w. A random sample of the plants with yellow anthered phenotypes and their phenotypes at the different dates are given in Table 3 along with the identified genotypes from Table 7. From Table 3 it is apparent that anther classifications varied considerably between dates for several of the individual plants.

A complementary factor controlling the expression of fullness of anthers was indicated during the June 29, 1954, reading and re-evaluated at subsequent readings. This new gene locus has been designated the *Sh* locus for the generally shrunken anthers of the homozygous recessive phenotype.

Table 4.—Chi-Square Tests of 15-1-7E2 Progeny by Dates of Classification Fit to a 3:1:4 Ratio.

Date	Class	Observed	Calculated	P Values
6-29-54	$1\text{-}4_y$	47	48	
	5_y	19	16	
	6_w	62	64	70-80%
		128	128	
8-27-54	$1\text{-}4_y$	42	45.38	
	5_y	19	15.13	
	6_w	60	60.50	
		121	121.01	50-70%
2-23-55	$1\text{-}4_y$	41	41.25	
	5_y	17	13.75	
	6_w	52	55.00	
		110	110.00	50-70%
3- 3-55	$1\text{-}4_y$	46	44.25	
	5_y	19	14.75	
	6_w	53	59.00	
		118	118.00	30-50%
Over-all Category	$1\text{-}4_y$	51	46.5	
	5_y	17	15.5	
	6_w	56	62.0	
		124	124.0	50-70%

Depending on the genotypes of the parents, the presence of the *Sh* gene may change the expected backcross ratio of 1:1 for the *X-x* locus to a ratio of 3 yellow full: 1 yellow shrunken: 4 white or a ratio of 1 yellow full: 1 yellow shrunken: 2 white. For example, S *xxShsh* by N *XxShsh* gives a 3:1:4 ratio because *Sh* fails to act in the presence of *xx* in S cytoplasm and for the same reason S *xxShsh* by N *Xxshsh* gives 1:1:2 ratio. The data in Table 4 show a good fit to a 3:1:4 ratio indicating the genotype of the female parent of I5-1-7E2 to be S *xxShsh* and that of the male parent to be N *XxShsh*.

To further test the action of the *Sh* gene, the progenies from open pollinated white anthered plants (classified 6_w at all readings) in the isolation plot containing the 15-1-7E2 progeny were indexed in the spring of 1955. Since the only source of pollen in this plot was from the yellow dehiscing anthers, the genotypes of the white anthered females could be determined, assuming even distribution of pollen, from the segregation ratios observed in plants grown from the open pollinated seed. The derivations of the ratios for each genotype are given in Table 5.

Table 5.—Derivation of Ratios Expected From Sib-Pollination of White Anthered Plants in I5-1-7E2 Progeny with the Yellow Dehiscing Anthered Plants in the Same Progeny.

Parents						Offspring
Phenotypes	Genotypes					
Male	Male Gametes					
1_Y-4_Y	1 S XxShSh	1(2 XSh : 2 xSh)				
1_Y-4_Y	2 S XxShsh	2(1 XSh : 1 xSh : 1 Xsh : 1 xsh)				
5_Y	1 S Xxshsh	Pollen Production Negligible				
	Male Gamets 4 XSH	4 xSh	2 Xsh	2 xsh		
Female	Female Gametes	Genotypes of Progeny				Phenotypic Ratios Expected
6_w 1 S xxShSh	xSh	4XxShSh	4xxShSh	2XxShsh	2 xxShsh	1(1_Y-4_Y):1(6_w)
6_w 2 S xxShsh	xSh	4XxShSh	4xxShSh	2XxShsh	2 xxShsh	
	xsh	4XxSh sh	4xxShsh	2Xxshsh	2 xx shsh	5(1_Y-4_Y):1(5_Y):6(6_w)
6_w 1 S xxshsh	xsh	4XxSh sh	4xxShsh	2Xxshsh	2 xx shsh	2(1_Y-4_Y):1(5_Y):3(6_w)
5_Y* ? S Xxshsh	Xsh	4XXShsh	4XxShsh	2XXshsh	2 Xxshsh	
	xsh	4XxShsh	4xxShsh	2Xxshsh	2 Xxshsh	7(1_Y-4_Y):2(5_Y):3(6_w)

* 5_Y appears as 6_w under certain conditions.

Since the size of progeny required to differentiate the expected ratios was large, classification was completed for only six of the open pollinated white anthered progenies. Of these six progenies, four fit the 5:1:6 ratio for S *xxShsh* mother plants and two fit a 2:1:3 ratio for S *xxshsh* mother plants. The chi-square tests for these ratios are given in Table 6.

Table 6.—Chi Square Tests of Open-Pollinated Progenies from White Anthered Plants. Plants.

Plant No.	Best Fitting Ratio	Observed	Calculated	P Value
43	5 (l_y-4_y)	45	43.75	90-95%
	1 (5_y)	8	8.75	
	6 (6_w)	52	52.5	
		105	105.00	
105	5 (l_y-4_y)	46	40.415	
	1 (5_y)	9	8.083	
	6 (6_w)	42	48.498	
		97	96.996	30-50%
104	5 (l_y-4_y)	45	37.5	
	1 (5_y)	7	7.5	
	6 (6_w)	38	45.0	
		90	90.0	20-30%
49	5 (l_y-4_y)	53	42.5	
	1 (5_y)	9	8.5	
	6 (6_w)	40	51.0	
		102	102.0	5-10%
37	2 (l_y-4_y)	31	32	
	1 (5_y)	15	16	
	3 (6_w)	50	48	
		96	96	90-95%
46	2 (l_y-4_y)	20	31.668	
	1 (5_y)	22	15.834	
	3 (6_w)	53	47.502	
		95	95.004	2-5%*

* Plant 46 occurred in the isolation plot next to plant 47 later identified as a SXx SHsh genotype. which, when crossed to a Sxx shsh plant, would give a 1:1:2 ratio very close to the observed ratio.

An additional test of the interactions of the *X-x* and *Sh-sh* alleles was obtained when the selfed progenies of the yellow anthered I5-1-7E2 plants were classified in the greenhouse during the winter of 1955-56. Sufficient plants were obtained from 15 of the 38 lots of selfed seed to index them for another type. The results of this classification are given in Table 7. Three of the progenies had parents of the S*XxShSh* genotype; ten of them had parents of the S *XxShsh* genotype; and two progenies had parents

(Plants 12 and 61) of the S *Xxshsh* genotype. The anther classification of plant 12 was 5_y (yellow shrunken), in the first two readings, and 3_y (yellow "full") in the last two. Plant 61 had an anther classification of 5_y in the first three readings and 4_y in the fourth. This evidence corroborates Experiment 1 in that the S *Xx* genotype, without the dominant *Sh* gene, has a variable phenotypic expression ranging from empty white to yellow, partly full and dehiscing anthers with viable pollen. This fact would also explain some of the low P values obtained for the selfed progenies of S *XxShsh* plants.

Table 7.—Results of Chi-Square Tests to Determine Genotypes from the Selfed Progeny of the Yellow Anthered Plants in the I5-1-7E2 Progeny.

Plant No.	Observed Ratio			Best Fitting Ratio			P Value	Probable Genotype
	1_y-4_y	5_y	6_w	1_y-4_y	5_y	6_w		
21	36	0	11	3	0	1	80%	S Xx ShSh
51	21	0	5	3	0	1	50%	S Xx ShSh
55	97	0	29	3	0	1	80-90%	S Xx ShSh
9	28	4	14	5	1	2	50-70%	S Xx Shsh
17	32	1	8	5	1	2	5-10%	S Xx Shsh
47	63	8	22	5	1	2	30-50%	S Xx Shsh
53	54	2	21	5	1	2	2-5 %	S Xx Shsh
57	63	7	26	5	1	2	30%	S Xx Shsh
82	87	6	26	5	1	2	1-2 %	S Xx Shsh
85	39	12	30	5	1	2	2-5 %	S Xx Shsh
98	68	8	15	5	1	2	5-10%	S Xx Shsh
106	36	4	25	5	1	2	2-5 %	S Xx Shsh
113	14	3	12	5	1	2	10-20%	S Xx Shsh
12	12	8	21	1	"3"		50-70%	S Xx Shsh
61	9	3	13	1	"3"		30-50%	S Xx Shsh

The variable phenotypic expression of the S *Xx* genotype has made the task of indexing for true breeding type O (N *xx*) hermaphrodities very difficult, since the observed ratios in the progenies may be unreliable and also the genotype of the female parent of the progeny may have been S *Xx* instead of S *xx*. Although there is still some phenotypic variability in the yellow anthered plants containing the *Sh* factor, the presence of this factor seems to insure sufficient pollen in the anthers to avoid misclassification as a white anthered plant.

Anther classifications should not be made too early in the blooming period, since anthers of the early opening flowers may be nearly white even though subsequent flowers will have yellow full anthers. Some flowers have been observed to contain both white anthers and nearly full yellow anthers. White anthered plants used as females to index for type O plants should be checked several times to see that they remain white anthered plants.

The *Sh* allele may be valuable in cytoplasmic male-sterile indexing work, since the progeny from the cross S *xxShSh* by N ..... should not have the yellow shrunken category which is difficult to detect. The *Sh* gene may be thought of as a complementary gene, since it will enhance pollen production if the genetic factor for pollen restoration is present in male-sterile cytoplasm.

Summary

A study of the phenotypic expression of various semi-male-sterile genotypes in cytoplasmic male-sterile sugar beets has revealed that the S *Xxshsh* genotype has a variable expression of anther color which leads to faulty phenotypic ratios.

A new genetic factor *Sh* is reported which, when present in the dominant condition, enhances the pollen-producing ability of the S *Xx* to such an extent that its classification as a white anthered phenotype is improbable.

Linkage between the *X-x* locus for pollen restoration in male-sterile cytoplasm and *M-m* locus for monogermness is indicated. However, pleiotropic action of the monogerm gene in the recessive *mm* genotypes to produce more white phenotypes is not precluded.

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Measurement of Salt Content of Beet Juices by Total Acidity

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The amount of ionic materials in sugar beet juice is an important factor in its processing quality. This has been recognized by the emphasis on ash and conductivity determinations. With the advent of stable, easily regenerated resins of analytical grade quality, another measure of salt content becomes available. This paper reviews the method used at this Laboratory and some of the findings obtained with it.

Materials and Method

About 10 ml. of wet resin or about 5 grams of air-dried resin (Dowex 50 or similar resin, mesh size about 60-100) was washed into a 12 mm. diameter tube stoppered at the lower end with a one-hole rubber stopper covered with fine-mesh nylon cloth. The resin was regenerated with 5 percent H_2SO_4 and washed with ion-free water until the pH of the effluent was 6 or above. Ten ml. of beet processing juice adjusted to about 10 percent sucrose was added to the column at a flow rate of about 10 ml./min. The column was washed with about 20 ml. of ion-free water or until the pH was 6 or above. The washings and eluate were made up to a definite volume, say 50 ml. Ten ml. of this solution were titrated with standard 0.1N NaOH to a phenolphthalein endpoint. Results may be expressed in milliequivalents of base per 100 grams of sucrose or other accepted units.

Results and Discussion

When determining the composition of beet processing juices, total available anions was used as a measure of the completeness with which the anions were accounted for. Thus it was proved that nearly all the acids in beet juice were accounted for as Cl, PO_4, citrate, SO_4, and malate (1). In diffusion juice lactic acid is an important factor when fermentation occurs. Recently in some unpublished work acetic acid was also found presumably as

Table 1.—Total Anionic Constituents in Beet Diffusion Juices as Affected by Storage.

Factory	Date of sample	Anionic const. ml./l.	Lactic acid ml./l.
	1953	10% sucrose basis	
Moorhead	Oct.	74	1.9
	Dec.	76	2.2
Brighton	Oct.	66	0.7
	Dec.	68	2.1
Toppenish	Oct.	42	1.1
	Dec.	45	1.5

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[2] Numbers in parentheses refer to literature cited.

one of the products of fermntation (2). In molasses lactic (3), glycolic (3), glyceric (3), acetic (2), and formic (2) acids were found to increase in quantity over that in the diffusion juice.

One of the simplest methods for following certain changes during storage of sugar beets is measurement of total acidity. Diffusion juices from portions of beets harvested in October were analyzed for total anionic constituents. Diffusion juices were obtained from the same factories and from similar beets in December and analyzed. The results are given in Table 1. It is apparent that there is an increase in acidic constituents. As much as half the increase was found to result from a lactic fermentation as shown in Table 1.

In the hope that salt content would show some relationship to sucrose percentage both were measured in a series of 50 beets by Dr. D. W. Greenwood, Utah State College, Logan, Utah. The total anions and sucrose showed a —0.15 correlation which is not significant. This is unexpected because sodium tends to show a marked negative correlation and potassium a negative correlation of lower significance. Anions should reflect any increase in cationic constituents so further examination of this apparent anomaly is suggested.

In recently reported work by J. Pomeranz and C. Lendner (4) an equation relating total anions to ash content of sugar products has been developed This equation is $y = 0.0570x - 0.00893$ where y = ash and x = ml. of N alkali used per 100 grams of sugar and offers a rapid test for the salt content of sugar.

Conclusion

Total anionic constituent determination is recommended as a reliable, precise and accurate method for following certain characteristics of progressing quality of beet juice and sugar products in the factory and should be examined as a tool to aid the plant breeder in eliminating low-sugar beets.

References

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